ANTI-INFLAMMATORY THERAPY IN ALLERGIC AIRWAYS DISEASE

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Dedication

To my wife, Erika, my brother, David, and my parents for their support, encouragement and love.

Declaration

I declare that this thesis has been composed by Dr Andrew Malcolm Wilson and comprises work performed by myself between August 1996 and October 1998 at the Department of Clinical Pharmacology, Ninewells Hospital, Dundee, UK. The study in Chapter 9 was the result of collaborative work with my colleague, Dr Imran Aziz. All of the other chapters contain studies, which have been designed, conducted, analysed, and written by myself. This thesis has not been submitted for any other degree, diploma or professional qualification.

Acknowledgement

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Abstract

The systemic adverse effects of inhaled corticosteroids were investigated in dose-response studies. By measuring cortisol suppression, it was shown that a more potent inhaled corticosteroid (fluticasone propionate (FP)) exhibits greater systemic bioactivity (2-fold at highest licensed doses) than a weaker steroid (triamcinolone acetonide (TAA)). No differences were detected between inhaled corticosteroids of similar potency (TAA and flunisolide), even when using sensitive and novel measures e.g. low dose ACTH stimulation and early morning urine cortisol excretion. The latter test may prove to have clinical implications for monitoring patients, as it was shown to be more sensitive than dynamic or basal serum cortisol measures. However, the lung delivery of a corticosteroid has a greater effect on systemic bioactivity than its dose or potency, as the systemic activity of FP via two different inhaler devices was shown to vary more than 5-fold. Studies comparing oral prednisolone with inhaled FP, showed FP to exhibit dose-related suppression of serum cortisol in a 1:8.5mg ratio compared to prednisolone. Interestingly, the effects of FP on markers of bone metabolism were less marked than adrenal suppression, compared to the effects of prednisolone. Intra-nasal FP also produced significant urinary cortisol suppression, whereas other intra-nasal corticosteroids (TAA, budesonide (BUD), beclomethasone, and mometasone) had no significant effects on 24 hour cortisol, bone or blood markers. Furthermore, the addition of intranasal to inhaled FP resulted in more patients with sub-normal cortisol values.

When assessing therapeutic effects of inhaled corticosteroids, it was shown that a lower dose of BUD was required to optimise symptoms, lung function and exhaled nitric oxide (NO), compared to other surrogate markers of inflammation such as serum eosinophilic cationic protein (ECP), and bronchial hyperreactivity to adenosine monophosphate (AMP) and methacholine. Thus airway inflammation may be inadequately controlled by titrating steroid dose according to lung function alone. However, higher doses of BUD resulted in greater adverse effects and a lower therapeutic index.

The anti-inflammatory effects of steroid sparing agents were, therefore, investigated. A leukotriene receptor antagonist, montelukast (MON), was less effective than inhaled plus intra-nasal BUD as monotherapy in patients with allergic rhinitis and asthma, in terms of airway hyperreactivity to AMP challenge, nasal and exhaled NO, and there was a trend towards lesser effects with symptoms, peak expiratory and nasal inspiratory flow rates. MON, however, did demonstrate anti-inflammatory activity in terms of exhaled NO and AMP challenge. In a similar comparison of BUD and the long acting β_2-agonist, formoterol (FM), there were no anti-inflammatory effects seen in terms of AMP challenge, eNO or ECP with FM. However lung function improved equally with both therapies and indeed patients preferred FM therapy. The combination of BUD and FM conferred additive effects. Finally, the effects of a long-acting β_2 agonist (salmeterol) and MON were compared as second-line therapy in asthmatic patients not controlled on inhaled corticosteroids. This demonstrated similar improvements in lung function and symptom control with both drugs but MON seemed to have greater anti-inflammatory effects in terms of AMP challenge and blood eosinophil count.

Abbreviations

μg	microgrammes
ACTH	Adrenocorticotrophic hormone
AMP	Adenosine monophosphate
AUC	Area under curve
AUC_{0-24}	area under the curve between 0 and 24 hours
BDP	Beclomethasone dipropionate
bid	twice daily
BUD	Budesonide
CRF	Corticotropin releasing factor
DEXA	dual-energy X-ray absorptiometry
DICE	Dose of Inhaled Corticosteroids with Equisystemic Effects
DNA	deoxyribonucleic acid
DP	Dry Powder
ECP	Eosinophilic cationic protein
FACET	Formoterol and Corticosteroids Establishing Therapy
FEF_{25-75}	Forced expiratory flow between 25% and 75% of forced expiratory volume
FEV_1	Forced expiratory volume in 1 second
FM	Formoterol
FN	Flunisolide
FP	Fluticasone propionate
FVC	Forced vital capacity
GCR	glucocorticoid receptor
GM-CSF	Granulocyte/macrophage colony stimulating factor
H	High
hCRF	Human corticotropin releasing factor
HPA	Hypothalamic-pituitary-adrenal
hrs	hours
IgE	Immunoglobulin E
IL	Interleukin
KPa	kilo Pascal's
L	Low
M	Medium
MANOVA	Multifactorial analysis of variance
MCh	Methacholine
MDI	metered dose inhaler
MF	Mometasone furoate
mg	milligrammes
mRNA	messenger ribonucleic acid
NF-κB	Nuclear factor kappa B
NO	Nitric oxide

od	once daily
PC_{20}	Provocation concentration causing fall in FEV_1 of 20%
PD_{20}	Provocation dose causing fall in FEV_1 of 20%
PEF	Peak expiratory flow
PEFR	Peak expiratory flow rate
PIFR	Peak inspiratory flow rate
PL	Placebo
pMDI	pressurised metered dose inhaler
Pred	Prednisolone
psi	pounds per square inch
qds	four times per day
RAST	Radioallergosorbent testing
RIA	Radio-immuno assay
SE	Standard error
TAA	Triamcinolone acetonide
Th	T-helper lymphocyte
Th0	T-Helper 0 Lymphocytes
Th1	T-Helper 1 Lymphocytes
Th2	T-Helper 2 Lymphocytes
TNFα	Tumour necrosis factor α

CHAPTER 1

INTRODUCTION

1.1 AIRWAYS INFLAMMATION

Allergic diseases result from an exaggerated immune response, on exposure of harmless environmental antigens, in previously sensitised individuals. Examples of such antigens include house dust mite, pollens and animal dander. The term allergy means "altered working" and was first used in 1906 by Von Pirquet to describe the fatal reaction that dogs, immunised with venom proteins from another animal, had after another injection of that protein[1]. The 'alteration' in response was that the dogs had an adverse effect (anaphylaxis) rather than the normal prophylactic response to immmunisation. More recently is has been realised that the allergic response is an immunoglobulin E (IgE) mediated or type 1 hypersensitivity reaction.

Individuals who have a tendency to develop such a response are described as being atopic. It is well recognised that atopy runs in families although the precise inheritance has currently not been fully evaluated. Bodner et al[2] have shown in a case control study that the onset of wheezing is related to atopic status and family history of atopic disease, with relative risk of 3.28 and 5.49 respectively. Atopy is usually diagnosed by the presence of a positive reaction on skin prick testing with common allergens[3], and in this respect, positive skin prick tests and specific IgE levels are associated with underlying asthma[4,5]. The association is more striking when assessing skin prick or radioallergosorbent testing (RAST) to house dust mite[6,7].

Although allergic diseases encompass conditions such as eczema, urticaria, food and

venom allergies, it is the involvement of the upper and lower airways (allergic rhinitis and asthma) which are the most common, and will be the focus of the work in this thesis. Asthma is defined as a condition of widespread narrowing of the bronchial airways changing in severity over short periods of time, either spontaneously or with treatment[8]. Allergic rhinitis, however, is characterised by sneezing, rhinorrhoea, nasal congestion and pruritis of nose and eyes with a temporal correlation to exposure of allergen[9]. Often included in the description of both conditions is a reference to inflammation and mucosal hyperresponsiveness.

The prevalence of asthma and allergic rhinitis is increasing[10,11] and worldwide, up to 20% of young adults have asthma[12]. The United Kingdom has one of the highest prevalence rates[13], where there are more than 100,000 admissions to hospital per year[10]. The prevalence of rhinitis is more difficult to calculate as many patients are self-treating, however, in adults it may be as high as 20%[12,14]. Although few patients with rhinitis require hospitalisation, it results in impairment in the quality of life of sufferers.

As mentioned above, patients with allergic airways disease produce specific IgE to inhaled allergens. After exposure to an allergen, antigen presenting cells (e.g. macrophages and dendritic cells) present immunogenic information to T-lymphocytes in association with major histocompatibility complex class II molecules, which in turn communicate with B-lymphocytes[1,15]. Macrophages normally have an immunosuppressive action and are weak antigen presenting cells, in contrast to dendritic cells, although this seems to be altered in asthma[16]. It is now accepted that there are two

subsets of T helper lymphocytes (Th1 and Th2)[15,17]. Both Th1 and Th2 develop from an initial common state (Th0). Th1 cells produce interferon gamma and promote cell mediated immune responses, whereas Th2 produce interleukin (IL)-4, IL-5 and IL-13, and promote humoral immune responses. IL-12 and IL-18 promote Th1 cells, whereas IL-4 induces Th2 cells. It is thought that the balance between these two cell types is in favour of Th2 responses in allergic individuals[18].

High affinity receptors for IgE are found on mast cells and basophils whereas lymphocytes, monocytes and eosinophils possess low affinity receptors. The importance of mast cells in allergic rhinitis and asthma is evident by the increased numbers of these cells in nasal and lower airway mucosa after exposure to aeroallergen[19,20]. When antigen binds to two specific IgE molecules, which themselves are attached to mast cells receptors, cross-linking of the receptors occurs, producing degranulation of the mast cell. This results in a release of inflammatory mediators which are either pre-formed and stored in granules, or newly synthesised in the mast cell[21]. These inflammatory mediators induce a variety of processes including increased vascular permeability, which results in oedema, and swelling of tissues, airway smooth muscle contraction which results in bronchoconstriction, as well as infiltration of further inflammatory cells. Stimulation of nocioceptors in neural fibers may give rise to sneezing and coughing in rhinitis and asthma respectively. Mast cells can also be triggered independently of IgE, by changes in oncotic pressure as seen during exercise or pharmacologically by adenosine monophosphate (see sections 1.2.2 and 2.12.2). More recently mast cells have been shown have role in modulating the inflammatory response by secreting IL-4[22].

After challenge with antigen, patients with allergic rhinitis or asthma experience airway narrowing which occurs within a few minutes and lasts for about an hour. This is called the early phase reaction or type 1 immediate hypersensitivity reaction, and occurs as a result of the process outlined above. However, after a few hours there is a further (late-phase) response, which is considered to be due to an influx of inflammatory cells to the submucosa, and is associated with an increase in non-specific bronchial/nasal hyperreactivity. The submucosa becomes infiltrated with chronic inflammatory cells which release a plethora of cytokines (e.g. IL-1 to 5, IL-13), chemokines (e.g. IL-8, RANTES, MCP-3 and 4) and other mediators (e.g. platelet activating factor) acting to cause further increased production, migration, adhesion, priming and survival of inflammatory cells[23]. This late phase reaction can be attenuated by cromoglycate, which stabilises mast cells suggesting a mechanistic link between early and late phase reactions.

The eosinophil has been recognised to be associated with atopic airways disease for many years, with blood levels being higher in atopic individuals[24]. Increased numbers of eosinophils have been found in the pathology specimens of patients dying of acute asthma, and more recently, in the sputum and bronchoalveolar lavage of patients with asthma[25-27]. Furthermore, the increase in peripheral blood eosinophil count occurring 24 hours after allergen challenge is related to non-specific airway hyperresponsiveness[28]. Eosinophilic granule proteins, for example major basic protein, eosinophil peroxidase and eosinophilic cationic protein (ECP), have been shown to have cytotoxic effects on airway epithelium[29], and to activate other pro-inflammatory cells.

Eosinophils also contribute to the inflammatory process by releasing other mediators such as platelet activating factor, leukotriene C4 (both of which can cause bronchoconstriction and mucous secretion), and pro-inflammatory cytokines including IL-1, IL-3, IL-5 and granulocyte/macrophage colony stimulating factor (GM-CSF)[30].

Basophils, also possess IgE receptors, synthesise histamine, and are involved in the late phase of the allergic response. Neutrophils are present in the airways of patients with severe asthma and contribute to the inflammatory process although their presence is short-lived[31]. Indeed in patients with sudden fatal asthma neutrophilia may be greater than eosinophilia. The epithelium is also involved by expressing inflammatory cytokines, chemotactic factors and adhesion molecules[32].

The inflammatory process detailed above leads to damage and denudation of the airway epithelium. This, in turn, may result in exposure of sensory nerves, and give rise to sneezing and coughing. Epithelial cell damage will reduce the production of epithelial-derived relaxant factor and may exacerbate bronchoconstriction[31]. A characteristic result of mucosal inflammation and epithelial damage is hyperresponsiveness, or the tendency for airways to react to specific or non-specific stimuli at doses less than in the normal population. This can be seen in both asthma and allergic rhinitis (see section 1.2.2).

As well as inflammatory cell infiltration and inflammation, the pathogenesis of asthma also includes smooth muscle hypertrophy[33], thickening of airway wall due to

subepithelial fibrosis[34], increased vascularity and oedema formation[35]. All of these factors lead to airway narrowing, which is compounded by mucus secretion and plug formation as a result of goblet hypertrophy. Other non-inflammatory mechanisms such as lung hyperinflation also contribute to symptomatology in asthma.

It is evident that there is a similar pathophysiology between asthma and allergic rhinitis[36]. Indeed, the two conditions frequently co-exist[37-39]. Patients with allergic rhinitis have higher indices of lower airways inflammation compared to healthy controls[40-45]. Mechanisms of this phenomenon include a neural reflex between upper and lower airways[46-48], increased incidence of mouth breathing due to nasal obstruction[49], and post nasal drip of secretions from the nose into the trachea. As there is significant overlap in the pathophysiologies of allergic rhinitis and asthma, it is important to optimise the control of nasal inflammation when treating patients, in order to optimise control of lower airway inflammation[50]. Studies have shown that in patients with allergic rhinitis and concomitant mild asthma, intra-nasal corticosteroids as monotherapy prevent the increase in airway hyperreactivity during the pollen season[51,52], and improve asthmatic symptoms[53]. Also in patients with allergic rhinitis, orally inhaled budesonide, without nasal exposure, decreases nasal lavage ECP concentration[54]. There are obvious anatomical differences between the nose and lungs for example the nasal mucosa has greater capacitance blood vessels and no smooth muscle. Also the allergen may be different as pollen sensitisation is more common in rhinitis whereas allergy to house dust mite is more common in asthma[55].

1.2 MEASURES OF INFLAMMATION AND DISEASE CONTROL

1.2.1 Symptoms and lung function

The simplest method of assessing disease control is to determine the severity of patients' symptoms. This is main outcome measure in clinical practice. Clinical trials also assess treatment response by quantifying the symptoms using methods ranging from simple scoring to complex questionnaires. The commonest method is to ask patients to score their overall asthma or rhinitis symptoms on a scale e.g. between 0 and 3. More in depth assessment will quantify individual components of asthma or rhinitis symptomatology. For example, patients can be asked about their cough, wheezing, breathlessness or nasal blockage, sneezing, rhinorrhoea or itching eyes[56,57]. It is particularly important to assess nocturnal symptoms in asthma as well as nocturnal wakening. Validated general or disease specific quality of life questionnaires are also available[58-60]. Rescue usage with reliever medication can also be employed[61] as can exacerbation rates in large sample studies[62].

The most commonly used objective measure of asthma disease control is that of pulmonary function testing. Peak expiratory flow rate (PEFR) is the simplest method and can be measured at home by the patient using a non-expensive device. This has the advantage of assessing daily changes in measurements as well as diurnal variation, which is a sensitive marker of disease control[63]. However, Cote et al[64], reported that patients record only one third of readings after 1 year despite regular reinforcement. Furthermore, more than one quarter of patients fabricated their results most of the time.

Nasal function can also be measured simply by a peak nasal inspiratory flow rate but until recently this is not routinely performed in the domiciliary setting[65].

Laboratory measures of lower airways function include spirometry and airways resistance[66]. The most common measures being forced expiratory volume in the first second of expiration (FEV_1) and mid expiratory flow rate (FEF_{25-75}). These have the advantage of reflecting changes in smaller airway caliber, which is more relevant in asthma. Airways resistance is determined by the ratio of the pressure difference between the mouth and alveolus to the air flow. Nasal obstruction, an important feature of seasonal allergic rhinitis[14], can be measured in the laboratory by acoustic rhinometry and rhinomanometry. The former measure assesses geometric changes by quantifying nasal volume and cross sectional area, whereas the latter measures function in terms of nasal resistance. Both of these parameters have been validated as sensitive measures of assessing rhinitis[67,68]. However, as the equipment required for these laboratory measurements is often bulky and expensive, they are rarely performed in the domiciliary setting and therefore day to day variation cannot be determined.

1.2.2 Challenge testing

Hyperresponsiveness is a characteristic feature of both allergic rhinitis and asthma. It is related to the degree of underlying mucosal inflammation[69,70] and correlates well with disease severity, requirement for therapy[71,72], and other markers of inflammation. Studies have shown that after allergen inhalation there is eosinophilic airway inflammation and associated increase in bronchial hyperresponsiveness[26,73]. Also, after

viral infection there is an inflammatory infiltrate and hyperresponsiveness[74].

In order to quantify the degree of hyperresponsiveness, an airways (either bronchial or nasal) challenge test is performed[75]. Increasing amounts of a chemical or physical stimulus is applied to the mucosa until a predetermined response is detected. This is normally in terms of airway narrowing such as nasal resistance, spirometry (e.g. FEV_1) or airway resistance. The stimuli produce their response either by *directly* acting on the airway, or *indirectly* by inducing the release of inflammatory mediators which, in turn, result in airway narrowing. Examples of stimuli acting *directly* are histamine and methacholine, whereas allergen, adenosine monophosphate, cold air, and exercise all act *indirectly*. When using pharmacological stimuli, the concentration or dose of drug required to cause a fall in FEV_1 of 20% is usually calculated (PC_{20}, or PD_{20} respectively).

In terms of bronchial challenge testing, direct stimuli are regarded as the gold standard[8]. Methacholine bronchial challenge has recently been shown to correlate significantly with eosinophil numbers from induced sputum, airway biopsies and bronchoalveolar lavage in asthmatic patients treated with inhaled corticosteroids[76]. In a study of children, responders to methacholine amounted to 98% of asthmatic children and 37% of healthy children[77]. However, adenosine monophosphate bronchial challenge, which causes bronchoconstriction indirectly by inducing the release of inflammatory mediators from primed mast cells[78,79], has been shown to be more sensitive in detecting anti-inflammatory effects and is probably more clinically

relevant[80,81].

Airway hyperresponsiveness may not only reflect the degree of airway inflammation[69]. Although studies have shown correlations with hyperresponsiveness and inflammation, there are other studies showing no association with bronchial hyperresponsiveness and inflammatory changes in sputum, bronchoalveolar lavage or biopsy[82]. Also, in the studies showing significant correlation, the correlation coefficient tends to be in the region of 0.6 indicating that other factors are involved. These include airway remodeling, bronchial smooth muscle contractility, airway compliance and lung elastic recoil[83,84].

There is a unimodal distribution of airways hyperreactivity in the population with asthmatic patients representing one end of the spectrum[85,86]. Therefore an arbitrary cut off value for the diagnosis of asthma is used. Most commonly a histamine PC_{20} of 8mg/ml is taken as the limit of normality. However, this will include up to 30% of healthy individuals[87]. Furthermore, as asthma is a condition which varies in severity, the degree of bronchial hyperresponsiveness will vary on a day to day, and month to month basis[88]. The variability of the test is such that the dose required to cause the required effect can vary up to 2-fold (or one doubling dose) in a given stable individual[89]. Bronchial challenge testing, therefore, is currently not often used to monitor an individual patient. However, it is a useful research tool for investigating the response to treatment.

1.2.3 Nitric oxide

Nitric oxide concentration is increased during airways inflammation by "up-regulation" of its generating enzyme, inducible nitric oxide synthase, by cytokines, including nuclear factor kappa B (NF-κB), IL-1β, and TNF-α which are generated in the inflammatory cascade[90]. Nitric oxide may be involved in the inflammatory process by increasing mucosal blood flow and plasma exudation[91]. Measuring the concentration of nitric oxide in exhaled or nasal air is a simple, non-invasive and reliable measure of the activity of this enzyme which in turn may reflect the degree of inflammation[92-98]. It has been found to be particularly useful in investigating the effects of steroids, as the activated glucocorticoid receptor complex inhibits the induction of this enzyme by inhibiting NF-κB[99]. Exhaled nitric oxide levels have been shown to be higher in untreated asthmatic patients[96,100] and to be reduced when treated with corticosteroids[101] but not with bronchodilators[102]. The level of exhaled nitric oxide has been shown to increase after bronchial challenge[103] and during acute exacerbations of asthma[104]. It also correlates well with sputum eosinophil counts in patients with asthma[105]. Nitric oxide has also been shown to be elevated in the nasal passages of patients with allergic rhinitis and to be suppressed with intra-nasal corticosteroid therapy[97].

Although nitric oxide production has been shown to be suppressed by anti-inflammatory therapy other than corticosteroids, for example with pranlukast[106] and montelukast[107], it seems to be particularly sensitive to corticosteroid therapy. This is probably because

the activated glucocorticoid receptor avidly inhibits the induction of nitric oxide synthase by inactivating NF-κB which is an important inducing cytokine of nitric oxide synthase[99]. Using nitric oxide alone to compare different forms of anti-inflammatory therapy may therefore lead to problems with interpretation as, for example, inhaled corticosteroids may have a greater effect than leukotriene receptor antagonists on exhaled nitric oxide for a given anti-inflammatory effect on the airways.

The technique of measuring nitric oxide may also lead to problems. As the normal concentration of nitric oxide from the upper airways is 100 times higher than from the lower airways, contamination of lung nitric oxide may occur unless the soft palate is able to closed off the upper airway (see section 2.13.2). Furthermore it may not be possible to accurately measure nasal nitric oxide in patients with severe nasal blockage as the technique involves the analyser drawing air from one nostril at a constant rate.

1.2.4 Blood Markers

Investigators have used measurements of peripheral blood markers to infer the state of asthmatic and rhinitic inflammation[108]. Blood eosinophil counts are known to be higher in patients with inflammatory conditions as the process releases cytokines which increase the production of these cells from the bone marrow[109]. Both peripheral blood eosinophil count and their state of activity, as measured by serum ECP, are considered to be sensitive surrogate markers of asthmatic inflammation[108,110]. Serum ECP correlates well with bronchoalveolar lavage ECP, eosinophil cell concentration in bronchial

biopsies and asthma exacerbations[111,112]. Serum ECP levels have also been shown to be elevated in patients who are pollen sensitive during the pollen season[113]. However, in a study by Hoshino et al[114], serum ECP correlated with mucosal eosinophil numbers, but not with airway hypersensitivity, pulmonary function or symptoms. Furthermore, Kips and Pauwels[115] felt that ECP should not be used as a diagnostic aid, but could be used to follow up patients with asthma. More recently, Gruber et al[116] showed that patients with bronchial hyperresponsiveness to cold air or histamine had higher levels of serum ECP, although this was not statistically significant and there was no correlation with serum ECP and histamine PC_{20}.

1.2.5 Other measures

Other, more invasive techniques can be employed but have not been utilised in any of the studies in this thesis. They include nasal biopsy or bronchoalveolar lavage and bronchial biopsy obtained during fibreoptic bronchoscopy. An alternative to bronchoscopy studies is to induce a patient to produce sputum by inhaling hypertonic saline[117] which, although better tolerated, is unpleasant, can be contaminated by saliva and is representative of the more proximal airways only[118]. These techniques have been validated[117,119], although cannot be repeated in quick succession as this causes an increase in neutrophil counts[120,121], probably due to the inflammatory nature of the procedure[122]. As the smaller airways are important in asthma some investigators have performed endobronchial airway challenge tests, by instilling histamine down a bronchoscope and measuring airway resistance in the bronchi[123]. In general all

measures of airway inflammation or disease control are related although the correlations are moderate. It is likely that all the currently available methods assess slightly different aspects of asthma or rhinitis inflammation and therefore their results should be taken together to gain a fuller picture of allergic airways disease control.

1.3 CORTICOSTEROIDS

1.3.1 Molecular mechanisms of Corticosteroids

When corticosteroids bind to the glucocorticoid receptor complex in the cytoplasm they cause dissociation of proteins (e.g. heat shock protein) enabling the glucocorticoid receptor to enter the nucleus and bind the DNA at sites called glucocorticoid response elements. This results in "up" or "down" regulation of many genes which are involved in the inflammatory process and subsequently favorable synthesis of inflammatory proteins and cytokines[99,124].

The activated glucocorticoid receptor also alters the inflammatory process by binding to transcription factors which are activated by cytokines. For example, RANTES is regulated by activator protein 1 and NF-κB, both of which are blocked by corticosteroids[125]. As discussed above, this is also the mechanism whereby corticosteroids attenuate the production of nitric oxide in the airways, as NF-κB causes activation of inducible nitric oxide synthase (section 1.2.3).

Corticosteroids have been shown to reduce the numbers of inflammatory cells in the

airways by inhibiting their survival and recruitment. For example, cytokines required to prolong the existence of eosinophils and vascular adhesion molecules, which aid their infiltration into the mucosa, are both inhibited by corticosteroids. Corticosteroids also reduce the inflammatory action of these cells[126], reduce epithelial shedding, microvascular permeability[127], goblet cell hyperplasia, basement membrane thickness[128,129] and reduce airway hyperresponsiveness[130].

Asthma and allergic rhinitis are considered to be chronic inflammatory conditions and all patients have persistent underlying airways inflammation[131,132]. For this reason, an important aim of medical therapy should be to control the disease activity by treating the inflammatory process. In this respect, topical corticosteroids have been shown to be the most effective anti-inflammatory therapy in the treatment of both asthma and rhinitis[9,133] which is in keeping with the current asthma management guidelines[134-136]. For example, clinical studies have shown intra-nasal corticosteroids to be more clinically effective than either placebo or antihistamines[51,137-140]. Likewise inhaled corticosteroids have greater effect on asthma symptoms than theophylline[141], cromoglycate[142] or antihistamines[143]. However there are few data regarding the natural history of asthma and the long-term effects of corticosteroid therapy[144].

1.3.2 Pharmacokinetics

The topical potency of the basic corticosteroid nucleus is increased by substitution of an ester group (triamcinolone acetonide, beclomethasone dipropionate) or a halomethyl

carbothioates group (fluticasone propionate, mometasone furoate). Relative potency can be measured *in vivo* by the McKenzie Vasoconstrictor Test[145,146], which assesses the degree of skin blanching after topical application. It can also be determined by *in vitro* measurement of glucocorticoid receptor affinity and glucocorticoid receptor complex residency time using radio-labeled competition assays with homogenised lung tissue[147]. More recently Stellato et al[148] compared the *in vitro* potency of inhaled corticosteroids by assessing basophil histamine release, eosinophil viability, and expression of a vascular cell adhesion molecule in the human bronchial epithelial cells. The results of these methods all give results with the following rank order of potency: fluticasone propionate ≡ mometasone furoate > budesonide > beclomethasone dipropionate ≡ triamcinolone acetonide > flunisolide[148-154]. As it is not possible to directly extrapolate the effects of corticosteroids between different tissues, the McKenzie Vasoconstrictor assay may have limitations. However, *in vitro* methods also have limitations as, for example, it is known that inflammatory cytokines can modulate glucocorticoid receptor affinity[155]. Although potency is an important factor in determining anti-asthmatic efficacy, it also has a critical effect on systemic adverse responses.

Lipophilicity, as measured by reverse phase high performance liquid chromatography, also differs between available corticosteroids[156], with fluticasone propionate being more lipophilic that other corticosteroids. Highly lipophilic drugs have been shown to have greater tissue retention within the mucosa[157], and greater glucocorticoid receptor affinity[150,156], however, they also have a greater volume of distribution and systemic

tissue accumulation[158].

As a large proportion of inhaled medication is swallowed, the degree of first-pass hepatic metabolism greatly influences systemic bioactivity. In this respect, fluticasone propionate and mometasone furoate have virtually complete metabolism, whereas budesonide, triamcinolone acetonide and flunisolide have first pass-metabolism of 11%, 23% and 20% respectively[159-163]. However, thorough mouth rinsing after inhalation, which is a recommended technique as it reduces the adverse topical effects of oral candidasis and dysphonia, can also reduce the swallowed fraction. In theory, inhaled corticosteroids can be absorbed systemically via the buccal mucosa thus by-passing first-pass hepatic metabolism, although this is thought to have a negligible effect on the systemic bioactivity[164].

As well as potency, receptor affinity and residency time, lipophilicy, volume of distribution, and extent of first pass metabolism, the plasma elimination half life also influences the systemic adverse effects of inhaled corticosteroids. In this respect fluticasone propionate (14.4 hours) has a considerably greater plasma elimination half life than the other corticosteroids (beclomethasone monpropionate: 6.5 hours, triamcinolone acetonide: 3.6 hours, budesonide: 2.3 hours, flunisolide: 1.6 hours)[158-161,165,166]. The longer elimination half-life of fluticasone propionate will result in greater accumulation at steady state[158].

1.3.3 Lung and Nasal Delivery

It is important to consider the lung and nasal delivery of inhaled corticosteroids, as well as the above pharmacokinetic properties[167]. As modern inhaled corticosteroids have a high degree of first pass hepatic metabolism of the swallowed fraction, but no first-pass metabolism of the inhaled or intra-nasal fraction of the drug, lung or nasal delivery will influence the systemic bioactivity as well as the clinical efficacy[168,169]. Therefore, the effect of each patient-drug-device combination must be assessed when considering the relative therapeutic ratio of inhaled or intra-nasal corticosteroids[170]. In a dose-ranging study of 35 severe asthmatic patients, some of whom were prednisolone dependant, Toogood et al[171] showed that the same dose of inhaled budesonide (either 400μg or 1600μg per day) doubled the asthmatic efficacy in terms of change in FEV_1 when given with a spacer compared to a metered dose inhaler alone. Furthermore there was not the same effect on systemic measures and therefore this resulted in a greater therapeutic ratio.

The importance of the inhaler device is also illustrated by *in vitro* data. In a study using the Andersen impactor chamber, respirable fractions (percentage of particles less than 5μm in diameter) of fluticasone propionate and budesonide were compared when delivered by their respective metered dose inhalers or dry powder devices[172]. With the metered dose inhalers the respirable fraction was 35% with fluticasone propionate and 21% with budesonide, however, fluticasone propionate Diskhaler produced only 12% respirable particles compared to 40% with budesonide Turbuhaler. Thus fluticasone

propionate metered dose inhaler had greater lung delivery than budesonide but this situation was reversed when comparing the dry powder inhalers. It is therefore not possible compare these two drugs without taking account of the inhaler device.

1.3.4 Measures of the Adverse Effects of Inhaled Corticosteroids

1.3.4.1 Hypothalamic-Pituitary-Adrenal Axis

Although corticosteroids have potent anti-inflammatory properties, they are also associated with adverse effects. These include osteoporosis and altered bone metabolism, thinning of the skin and bruising, cataracts, diabetes and psychological abnormalities. There are also concerns regarding growth retardation in children. Due to the similarities between the glucocorticoid receptor in all bodily tissues[173], it has not yet been possible to separate these beneficial and adverse effects.

The hypothalamic-pituitary-adrenal (HPA) axis is a fundamental hormonal cascade regulating glucocorticoid production from the adrenal cortex. Corticotropin releasing factor (CRF) is produced in the median eminence of the hypothalamus in response to physical or emotional stress, secreted into the hypophysial portal system and then carried to the anterior pituitary gland where it induces adrenocorticotrophic hormone (ACTH) secretion. ACTH acting through a membrane bound receptor induces cyclic adenosine monophosphate production, which in turn induces the enzymatic cascade resulting in cortisol production from cholesterol. Exogenous corticosteroids, produce negative feedback by stimulating glucocorticoid receptors on the anterior pituitary and

hypothalamus with a resultant fall in cortisol production and, in the longer term, adrenocortical atrophy. The degree of HPA-axis suppression can therefore be used to measure the systemic bioactivity of inhaled and intra-nasal corticosteroids[174]. It can be assessed by either basal or dynamic measures of adrenal function[175].

Measurement of cortisol, the endpoint of the HPA-axis, can be used to assess the whole cascade and can be determined in blood, urine or saliva samples. Production of cortisol is in a pulsatile manner and also varies throughout the day according to a circadian rhythm[176]. For this reason a single sample of plasma cortisol is less able to reflect HPA-axis activity than repeated samples integrated with respect to time[177]. However, studies have used 8am or 9am serum/plasma cortisol samples as this coincides with the time of greatest secretion[178]. Preferably, samples should be precisely at 0800hrs in patients who have been lying supine in a relaxed environment, as the lack of cortisol suppression of inhaled corticosteroids compared to placebo in some studies may be attributable to non-controlled conditions[179,180]. Twenty-four hour integrated plasma cortisol measurements are time consuming and cannot be used in clinical practice although they are frequently employed in clinical trials[149,181-183].

Measurement of urinary cortisol excretion has the advantage of smoothing out the fluctuations of serum cortisol. Indeed 24 hour urinary cortisol excretion has been shown to be more sensitive than 8am serum cortisol at detecting the systemic bioactivity of inhaled corticosteroids[184]. However, a 24 hour sample of urine is difficult to obtain in an out-patient setting and, for reasons of compliance, fractionated samples have been

used. McIntyre et al[185] showed an overnight urinary cortisol collection (from 2200hrs to 0800hrs) was as sensitive as the full 24 hour collection when corrected for creatinine, and more sensitive than morning serum cortisol measurements. This is understandable when considering the diurnal circadian rhythm of cortisol secretion, with highest levels during the night and early morning. It may also be possible to use an 8am spot sample of urinary cortisol, however, at present there are little data on the reliability of this measure.

The actual measurement of urinary cortisol may be performed in different ways and therefore different values can be obtained from different laboratories. It is important to measure free cortisol and therefore an extraction process should be performed[186]. Measurements can then be made by radioimmunoassay or high performance liquid chromatography. The advantage of radioimmunoassay is that it is a relatively cheap, quick process, although some of the commercially available kits have cross-reactivity with prednisolone.

Dynamic adrenal function testing may be more clinically relevant than basal measures as these tests mimic the body's response to physiological stress. The most commonly used is the 250μg ACTH stimulation test given as a bolus or 6 hour infusion. However, this dose is considered to be supraphysiological and although it is routinely used in clinical practice in the diagnosis of Addison's disease, it may not be sensitive enough to determine subtle abnormalities in the HPA-axis induced by inhaled corticosteroids[175]. In this respect, a low dose (0.5μg) ACTH test has been shown to more sensitive that the

250μg ACTH test, and correlates well with the insulin stress test[187]. In a study by Broide et al[188], comparing the high dose and low dose ACTH test, a quarter of adults and children who were taking long-term maintenance inhaled corticosteroids had a sub-optimal response to low dose but a normal high dose ACTH response. Kannisto et al[189] assessed the effects of fluticasone propionate and budesonide in asthmatic children, after 6 months using the low dose (0.5μg) ACTH test. The test was abnormal in 9 out of 30 children receiving budesonide, but only 5 out of 30 in the fluticasone propionate group, which is in keeping with the finding of more growth suppression in the budesonide group. Unfortunately, the ACTH stimulation test is now contra-indicated in the UK data sheet (Synacthen, Ciba Laboratories, Horsham, UK) in patients with allergy and asthma because of occasional reports of hypersensitivity and fatal anaphylactic reactions. Hence for ethical reasons, at least in the United Kingdom, studies using ACTH stimulation are limited to healthy volunteers.

The insulin stress test is regarded as the gold standard pituitary function test. Hypoglycaemia stimulates the hypothalamus to secrete CRF which then activates the HPA-axis. Unfortunately the hypoglycaemia necessary for the test results in unpleasant side effects and it cannot be routinely used for screening purposes. The human corticotropin releasing factor (hCRF) stimulation test also examines the integrity of the whole HPA-axis and has been shown to be as sensitive as the insulin tolerance test[190]. However, unlike the insulin tolerance test it is not associated with unpleasant reactions and there have been no reported cases of anaphylaxis. Also, the 100μg hCRF test

produces more of a physiological response than the high dose ACTH stimulation test. On a negative side the hCRF test is very expensive when compared to other tests, and has not been used in many clinical studies.

Studies by Brown et al[184], Broide et al[188] and Grebe et al[191] have also shown that patients who have impaired basal tests of adrenal function also have subnormal stimulation tests. For example in the study by Broide et al[188] there was a positive correlation between the peak cortisol response to ACTH and the 24 hour urinary cortisone excretion. Furthermore those patients who had an abnormal ACTH test had significantly lower 24 hour urinary cortisol than those with a normal response.

1.3.4.2 Bone

Although there have been reported cases of Addisonian crisis in patients who have been exposed to stress at the same time as stopping their inhaled corticosteroid therapy[192,193], and it is recommended that patients on long-term high dose therapy may require supplementary oral corticosteroids during stress challenges, this is extremely rare[194]. However, an important long term adverse effect of inhaled corticosteroid therapy is steroid induced osteoporosis and the associated risk of fracture[195]. At present, as there is no way of directly correlating between the effects on one tissue and another[196], clinical studies need to be performed to assess the effects on bone metabolism.

Corticosteroids alter bone metabolism by a number of different mechanisms. The dominant effect is through suppression of the osteoblasts and alteration in the bone

multicellular unit[197,198]. There is also increased bone resorption under the influence of increased parathyroid hormone, as a result of steroid induced reduction in the absorption of calcium from the gastrointestinal tract and renal tubules[199] amd disruption of collagen metabolism, bone matrix formation and sex hormone balance[197]. The main sites of decreased bone density and osteoporosis are the vertebrae, ribs and pelvis as these areas contain mainly trabecular bone which is ten times more metabolically active than cortical bone[200]. However, inhaled corticosteroids may also increase bone density by increasing mobility and weight bearing activity.

Although dual energy bone densitometry is the standard method of diagnosing osteoporosis and risk of fracture[201-203], this measurement is time consuming, expensive and, as the changes take many years, long term studies are required to compare treatments. For these reasons only a few studies have utilised bone density to compare inhaled corticosteroids, and biochemical blood bone markers have been used as a surrogate[175,204,205]. However, Herrala et al[206] performed dual-energy X-ray absorptiometry (DEXA) at the lumbar spine and at the left proximal femur in non-smoking women with asthma treated with beclomethasone dipropionate 1mg per day, and found no difference after 1 year compared to healthy control subjects. Osteocalcin is a hormone produced only by osteoblasts and is a specific surrogate marker of their activity[207]. It is therefore is more specific than other measures such as urinary calcium excretion and plasma alkaline phosphatase, and more sensitive than urinary hydroxyproline[208].

Studies have shown greater suppression of osteocalcin with oral prednisolone than budesonide at therapeutically equivalent doses[208,209]. In a study by Bootsma et al[210], inhaled fluticasone propionate was also reported to have less effect on osteocalcin production than beclomethasone dipropionate for a given change in spirometry. Interestingly both drugs had no effect on urinary cortisol excretion. The fact that different inhaled corticosteroids were found to have different tissue sensitivity has important consequences as it may be possible to have a greater effect on the lung compared to the adrenal gland. However only one dose of each drug was investigated and the results may therefore reflect different sensitivities of the tests rather than the tissues themselves. More useful information would have been obtained from a dose-ranging study (see section 1.4.1).

There is evidence that the dose-response curve for osteocalcin is not linear with a plateau above 800μg of beclomethasone dipropionate[211], analogous to the effects of cortisol. There are no dose-response studies comparing the effects of two different inhaled corticosteroids in terms of osteocalcin suppression. Also there are no data regarding intra-nasal corticosteroids and osteocalcin.

Furthermore, there is not sufficient information as yet to determine whether changes in osteocalcin level directly correlates with results of long term bone densitometry studies. It may only reflect recent treatment with inhaled corticosteroids[212]. Sorva et al[213] showed 8% suppression of osteocalcin in asthmatic children with inhaled budesonide

after 1 month of therapy and a similar level after 5 months. However, Hanania et al[214] found the product of dose and duration of inhaled corticosteroids, when corrected for body mass index, correlated to bone density measurements and adrenal suppression but not to osteocalcin levels. Osteoclacin did not correlate with the bone density. Urinary and serum break-down and formation proteins from collagen metabolism are also used[215], although these have not been investigated in this thesis.

1.3.4.3 Other measures of adverse effects of inhaled corticosteroids

As hypercortisolism results in oesinopenia, suppression of peripheral blood eosinophil count can been used to compare corticosteroids[175,196,216]. However, Wood et al[109] showed that after allergen challenge there was an increase trafficking of eosinophils from the bone marrow to the lung in dogs suggesting that suppression of blood eosinophil counts may reflect a reduction in the inflammatory response rather than a systemic adverse effect. There are other methods of assessing the systemic adverse effects of inhaled corticosteroids which have not been utilised in this thesis including determining eye changes in terms of cataract formation and ocular pressure, bruising and skin thinning, and growth in children measured by knemometry[175,195,217]. Ferguson et al[218] compared fluticasone propionate 400μg per day and budesonide 800μg per day in moderately severe asthmatic children. They showed that growth was reduced with budesonide, however, there was no suppression of adrenal function.

1.4 COMPARISION BETWEEN DIFFERENT CORTICOSTEROIDS

1.4.1 Requirement for dose-response studies

It is widely accepted that, for a given level of clinical efficacy, inhaled corticosteroids do not exhibit the same degree of adverse effects as oral corticosteroids. Comparisons have also been made between different inhaled corticosteroids, however, there is still doubt about their relative degree of systemic activity.

In a review article, Barnes et al[124] discuss the various study designs used to compare inhaled corticosteroids. The most valuable designs were felt to be dose-response comparisons, where at least two doses of each drug is compared on the steep part of the dose response-curve, or dose down-titration comparisons, where the doses of two drugs are reduced to the lowest clinically effective dose. These are the only methods by which potency ratios can be calculated. Of the two published dose-response studies comparing two different corticosteroids in terms of clinical efficacy, one compared budesonide and beclomethasone dipropionate[219] and the other compared beclomethasone dipropionate and fluticasone propionate[220]. However, in neither study was there a dose response effect of either drug and neither looked at systemic adverse effects.

There is one study which has compared the clinical efficacy of two inhaled corticosteroids using a back titration design[221]. The minimal dose of fluticasone propionate and budesonide, when given by their dry powder devices, required to control symptoms in asthmatic children was shown no different between the two corticosteroids.

This result may have been confounded by the fact that the minimum dose of each drug was 100μg per day, as it was not possible to administer a dose lower than this. In theory titration should have been continued down to zero, as there may have been differences between the drugs at lower doses. However the majority of patients (75%) had an exacerbation of asthma and the same findings were apparent when the analysis was performed only on those patients.

A less satisfactory alternative is to perform a dose-ranging study of one drug and compare that to one dose of another drug. This was the method employed by Dahl et al[222]. They concluded that 400μg per day of beclomethasone was equivalent to 200μg per day of fluticasone propionate. However, there was no dose response effect of fluticasone propionate and no significant difference between beclomethasone dipropionate and fluticasone propionate at 1600μg per day.

For efficacy studies the steroid sparing potential of inhaled corticosteroids can be investigated. Nelson et al[223] showed oral prednisolone to be eliminated in 75%, 89% and 9% of patients receiving fluticasone propionate 1mg, 2mg and placebo respectively; and Noonan et al[224] showed 69%, 88% and 3% elimination with 0.75mg, 1mg and placebo.

Barnes et al[124] felt that comparing one dose of two drugs had little or no value in establishing the potency of drugs. However, Pauwels et al[225] compared fluticasone

propionate 250μg per day and beclomethasone dipropionate 500μg per day and showed no difference in clinical efficacy, however fluticasone propionate had better adverse effects in terms of bone density and osteocalcin. Lorentzen et al[226] compared fluticasone propionate 1mg per day versus beclomethasone dipropionate 2mg per day and showed fluticasone propionate to have better effect on spirometry than beclomethasone dipropionate. Ayres et al[179] showed that after 6 weeks of treatment, fluticasone propionate at doses of 1mg per day and 2mg per day had a change in peak expiratory flow rate of 21 and 24 l/min respectively, and budesonide at 1.6mg per day had a 13 l/min improvement. However, there was a difference of only 3 l/min between the two doses of fluticasone and no dose-response effect. Barnes et al[180] showed fluticasone propionate 1mg had similar effects to beclomethasone dipropionate 2mg in terms of cortisol and peak expiratory flow rate. Leblanc et al[227] compared fluticasone propionate at 200μg per day with beclomethasone dipropionate 400μg per day. Both drugs had a similar improvement in peak expiratory flow rate, but fluticasone propionate had less effect on cortisol suppression.

However, Boe et al[228] compared fluticasone propionate 2mg per day with budesonide 1.6 mg per day and showed similar clinical efficacy, although fluticasone propionate had greater cortisol suppression. The data from this last study could be used to conclude that budesonide has a greater therapeutic ratio. However, this interpretation would be in contrast to the results of a meta-analysis performed by Barnes et al[229]. For this reason, single dose comparisons of inhaled corticosteroids at unknown points on the dose

response curve afford little information regarding the relative effects of inhaled corticosteroids. It is necessary therefore to compare inhaled corticosteroids using sensitive markers in appropriate patients at doses on the steep part of the dose-response curve.

1.4.2 Dose-response comparisons for systemic effects

1.4.2.1 Adrenal suppression

Of all the dose-response studies for adrenal suppression comparing two inhaled corticosteroids, fluticasone propionate and budesonide have been investigated the most. Donnelly et al[181], Grahnen et al[182] and Boorsma et al[183] have all shown dose related suppression for both drugs with greater suppression with fluticasone propionate in terms of 24 hour area under the curve (AUC) plasma cortisol, when compared on a microgram equivalent basis in healthy adult volunteers. Granhen et al[182] compared the drugs delivered by their respective dry powder devices while the other authors assessed these steroids delivered by a pressurised metered dose inhalers. More recently Derom et al[230] compared the effects of 400μg and 2mg of fluticasone propionate versus 400μg and 1.6mg of budesonide via their dry powder inhalers in moderately severe asthmatic patients. Compared to placebo there was no suppression of serum AUC_{0-20} cortisol at low doses but 34% and 16% suppression with high dose fluticasone propionate and budesonide respectively. There was a significant improvement in FEV_1 with all active treatments compared to placebo. Clark et al[231], in two separate dose-response studies comparing fluticasone propionate and budesonide given by metered dose inhalers,

showed greater suppression with fluticasone propionate in asthmatic adults, using overnight urinary cortisol, after single[232] and steady-state[231] dosing. With regard to children, Clark et al[233] performed in a dose-response study comparing 400μg, 800μg and 1250μg of inhaled budesonide and fluticasone propionate. Suppression with fluticasone, but not budesonide occurred at all doses. Lipworth et al[234], however, showed no suppression in a chronic dosing study with 400μg per day of either drug. The reason for the apparent discrepancy is that in the former study the total dose of the inhaled corticosteroids were given at night, whereas in the later the dose was given twice daily. The influence of a single dose on the HPA-axis is greater when given at night is greater than when given as divided doses, especially on measurements made the following day[235]. The only dose-ranging study to compare budesonide and beclomethasone dipropionate using 24 hour AUC plasma cortisol also showed budesonide to produce less suppression[149].

It is surprising that there is only limited published data regarding the HPA-axis effects of inhaled triamcinolone acetonide and flunisolide considering that, until recently, they were the most commonly used inhaled corticosteroids in the USA. Altman et al[236] performed a dose ranging study of triamcinolone acetonide at 800μg, 1200μg and 1600μg per day in asthmatic patients. Adrenal function was measured in terms of the 250μg ACTH test and 24 hour urinary cortisol after 2 weeks and 1, 3 and 6 months. There was no impairment of the dynamic stimulation test and the suppression of urinary cortisol was not out with the normal reference range. In a parallel group study of steroid

naive mild asthmatics, sequential cumulative doubling doses of triamcinolone acetonide (800-3200 μg per day) and flunisolide (1000-4000μg per day) were administered with dose increments at weekly intervals[161]. In terms of 24 hour uncorrected urinary cortisol excretion, triamcinolone acetonide had no detectable effect at 800μg per day, but produced 7% suppression at 1600μg per day, as compared to 13% and 15% suppression with 1000μg per day and 2000μg per day respectively of flunisolide. In another study of healthy volunteers, 3.5 days treatment with triamcinolone acetonide 2000μg per day and flunisolide 2000μg per day produced no significant effect on 24 hour urinary cortisol excretion[147].

There have been two dose-ranging studies comparing the relative effects of oral prednisolone and inhaled budesonide in terms of HPA-axis suppression. Toogood et al[196], in adult asthmatic patients, and Jennings et al[209], in healthy volunteers, showed dose related suppression with both drugs although there was greater suppression with prednisolone. There have been no dose-response studies comparing prednisolone with other inhaled corticosteroids.

While there have been some reports of systemic adverse effects with intra-nasal corticosteroids[237], other studies have shown no adverse effects[238-241] and these drugs are generally regarded as being completely safe. There have been no studies comparing the relative effects of different intra-nasal corticosteroids using sensitive markers of adrenal suppression.

1.4.2.2 Bone metabolism

Pauwels et al[225] showed that fluticasone propionate improved bone mineral density after 1 year, compared to beclomethasone dipropionate, and this was associated with higher serum osteocalcin levels. When comparing budesonide (1.6mg per day) and fluticasone propionate (1mg per day), Hughes et al[242] also showed an increase in bone density which was significantly related to change in osteocalcin levels after 1 year. In another study, no change in bone density, osteocalcin or morning cortisol with fluticasone propionate via a Diskhaler at 500μg twice daily after 2 years was found[243]. However, there was significant suppression of the 8hour 250μg ACTH test[243]. Gregson et al[244] compared beclomethasone dipropionate 200μg twice daily and fluticasone propionate 100μg twice daily for 20 months, and found no effect on bone density in children.

1.4.3 Dose-Response Relationships for Measures of Clinical Efficacy

There are only a few dose-response studies, which address clinical efficacy of intra-nasal corticosteroids, and it is unusual for clinicians to titrate the dose of corticosteroids for individual patients. The licensed dose is different for adults and children and there is usually an initial dose and a lower maintenance dose, but otherwise no dose alteration occurs. Dolovich et al[245] compared fluticasone propionate 200μg once daily with 200μg twice daily in patients with seasonal allergic rhinitis and found no difference for nasal blockage or rhinorrhoea although the patients taking the higher dose had significantly less nasal itching. A dose-response study with mometasone furoate 50μg,

100μg 200μg and 800μg per day for 1 month in seasonal allergic rhinitis showed clinical efficacy with all doses[246]. There was no advantage of 800μg per day over 200μg per day. However, the lower doses showed less activity after 1 week.

There is clinical and experimental evidence to suggest that the dose of inhaled corticosteroids required to adequately control asthmatic patients symptoms may not result in normal values for other methods of assessing clinical efficacy. In other words the dose-response curves for different measures of disease control will have different shapes. In a study by Pedersen and Hansen[247] moderately severe asthmatic children were given inhaled budesonide at 100μg, 200μg and 400μg per day. There was a dose-response relationship for exercise induced bronchoconstriction but a plateau in response after 100μg per day for symptoms and peak expiratory flow rate. In another study with fluticasone propionate there was a significant improvement between 100μg per day and 200μg per day in terms of methacholine bronchial challenge but not in terms of lung function (FEV_1, PEF) or asthma symptoms[248]. Toogood et al[249] showed that the shape of the dose-response curve depended on which measure of efficacy that was used.

Patient severity will also influence the dose-response curve, by shifting it to the left or right, and each patient will have their own dose-response curve for a given marker of efficacy. For example, in a study with beclomethasone dipropionate there was no improvement in terms of FEV_1 when increasing the dose from 400μg to 1600μg per day in patients who were controlled on inhaled corticosteroids[250]. Whereas in another study in more severe patients, requiring oral corticosteroids, a significant dose response effect

was seen when increasing the dose of inhaled beclomethasone dipropionate from 200μg to 1600μg[249]. This probably reflects the flat dose response curve in milder patients after 400μg per day but in more severe patients a plateau is not reached until at least 1600μg per day.

The duration of treatment also influences the dose response curve, as the time taken for each measurement to reach maximal response varies according to the endpoint chosen. For example, a clear response in terms of symptoms will occur before changes in lung function and airways hyperreactivity[251]. In general the longer the period of treatment the greater the chance of achieving the maximum effect. However, longer term studies are less likely to achieve good patient compliance. Furthermore, alterations in asthma control, due to the normal variability of disease severity, are more likely to effect long term studies. The majority of the effects of inhaled corticosteroids are achieved with most markers of efficacy in about 3 weeks[251,252].

Most dose-ranging studies have shown a statistically significant improvement between placebo and active treatment, regardless which inhaled corticosteroid was evaluated. Studies have shown dose-response effects for beclomethasone dipropionate[249,253] and budesonide[254-256] up to 1600μg per day; and fluticasone propionate up to 1000μg per day[257,258]. It is not possible to be certain about the shapes of the dose-response curves for different measures of efficacy, as there have been no dose-response studies comparing symptoms, lung function, serum markers and bronchial challenge tests in the

one group of patients.

1.5 SECOND-LINE THERAPY

Given the fact that all topical corticosteroids have adverse systemic effects at high doses it is important to ensure the dose is kept to a minimum. In patients not adequately controlled on low dose inhaled corticosteroids, second-line therapy may be introduced[134,259]. The purpose of introducing second-line therapy is to achieve adequate disease control without increasing the corticosteroid dose. As discussed above (see section 1.2) there are many ways of assessing optimal disease management, which include symptom control, normalisation of lung function and quality of life and absence of exacerbations of asthma. However, as asthma and rhinitis are inflammatory conditions, it is also important to consider the anti-inflammatory properties of second-line therapy as there is evidence that long-term untreated inflammation may lead to airway remodeling and irreversible airflow obstruction[260-262]. The aim of this thesis is to investigate the anti-inflammatory therapy in allergic airways disease and therefore this will be the main focus when considering the properties of second-line agents.

1.5.1 β_2-adrenoceptor agonists

β_2-adrenoceptor agonists, the most effective known bronchodilators, are divided into two classes according to their duration of action. Short-acting β_2-agonists exhibit their effects for 3-6 hours, whereas long-acting β_2-agonists (formoterol and salmeterol) last for more than 12 hours. As well as causing bronchodilation, β_2-agonists demonstrate

protection against bronchoconstricting stimuli such as exercise[263] and allergen[264]. This functional antagonism and long duration of action make long-acting β_2-agonists ideal candidates for second-line therapy in patients who were not adequately controlled on inhaled corticosteroids[134,135,265]. Furthermore, there is considerable evidence to suggest that long-acting β_2-agonists improve symptoms, exacerbation rates and lung function[62,266].

When prescribed in conjunction with inhaled corticosteroids, they have also been shown to be as effective as giving double the dose of inhaled corticosteroid alone. For example, Greening et al[267] performed a parallel study comparing 426 asthmatic patients who were randomised to either beclomethasone dipropionate 200μg twice daily plus salmeterol 50μg twice daily or beclomethasone dipropionate 500μg twice daily. After 6 months there was significantly greater improvements in morning and evening peak flow rate with the salmeterol plus beclomethasone dipropionate limb than the higher dose inhaled corticosteroids limb. More importantly, there was no difference in exacerbation rates between these treatment groups. Another study compared[268] the addition of salmeterol (50μg or 100μg twice daily) to beclomethasone dipropionate 500μg twice daily versus beclomethasone dipropionate 1000μg twice daily in asthmatic patients, also for 6 months. Similarly, there was greater improvements in morning and evening peak flow rates in patients receiving the long-acting β_2 agonist. There was no difference in exacerbation rates and neither treatment improved bronchial hyperresponsiveness to histamine. Pauwels et al[62] showed similar findings in the Formoterol and

Corticosteroids Establishing Therapy (FACET) study. This was a four way parallel study comparing budesonide 100μg twice daily, budesonide 100μg twice daily plus formoterol 12μg twice daily, budesonide 400μg twice daily, or budesonide 400μg twice daily plus formoterol 12μg twice daily. The addition of long-acting β_2 agonist was shown to reduce both mild and severe exacerbations when added to either the low or high dose corticosteroid.

It is uncertain as to whether long-acting β_2-agonists have anti-inflammatory activity. There is *in vitro* evidence that they have effects on mast cell degranulation, vascular permeability and inflammatory cell infiltration[269]. A recent biopsy study[270] showed inhaled formoterol to reduce the numbers of submucosal mast cells and eosinophils, although the baseline values were not equal and there was an out-lying value which may have influenced the results. Serum ECP levels have also been shown to be reduced with inhaled salmeterol[271].

There is a body of evidence that long-acting β_2-agonists may be detrimental in the long term care of asthmatic patients. The concern is that, as their main action is at the bottom of the inflammatory cascade, they may be treating symptoms and not inflammation. Indeed they may mask the underlying inflammation, resulting in a delay in treatment of asthmatic exacerbations[272]. This is in keeping with the results from the FACET study[62], which showed that although the combination of high dose inhaled budesonide and formoterol achieved the best control of asthma. High dose (800μg per day)

budesonide as monotherapy was superior to low dose (200μg per day) budesonide plus inhaled formoterol in terms of severe asthmatic exacerbation rates. There is also evidence to suggest that β_2-agonists may, in fact, inhibit the anti-inflammatory effects of corticosteroids on eosinophil survival in lung tissue[273], although other authors have shown that β_2 agonists do not compromise the ability of dexamethasone to suppress the generation of cytokines from monocytes[274].

Another concern with β_2-agonists is that they exhibit tolerance or tachyphylaxis to both their bronchodilator and bronchoprotective properties[275], which is measured by comparing the response after the first dose of long-acting β_2 agonist with the response after a prolonged period of treatment[276]. This effect is as a result of receptor down regulation and is more pronounced with long-acting β_2 agonists than with short-acting β_2-agonists due to the duration of receptor occupancy. The degree of tachyphylaxis is greater for the bronchoprotective than bronchodilator effects[275] and is more pronounced with indirect than direct stimuli[277]. In view of the therapeutic benefit of long-acting β_2 agonists in a large number of studies[266-268], it is felt by many physicians that tachyphylaxis is not clinically relevant. Although it has been shown that patients receiving long-acting β_2-agonists require more short-acting β_2 agonist to achieve a given degree of bronchodilation during an acute exacerbation of asthma[278].

Long acting β_2 agonists can be dispensed in either oral or inhaled preparation. The tablet formulation is obviously easier to administer although there is a greater incidence of

systemic adverse effects. The most common side effect is that of tremor, although they should be used with caution in patients with hyperthyroidism, ischaemic heart disease and arrhythmias. These adverse effects mean that some patients may not tolerate long-acting β_2 agonists.

1.5.2 Leukotriene receptor antagonists

Leukotrienes, originally referred to as slow-reacting substance of anaphylaxis, are important mediators in the inflammatory cascade. On release from the nuclear membrane by phospholipase A2, arachidonic acid is metabolised by the 5-lipoxygenase pathway to produce leukotriene A4. This is then metabolised via a cascade of enzymes to produce the cysteinyl leukotrienes – leukotriene C4, D4, and E4. These chemicals have been shown to have major effects in the pathophysiologies of rhinitis and asthma. They produce bronchial smooth muscle contraction, inflammatory cell chemotaxis, mucus hyper-secretion and neuronal stimulation[279]. Furthermore, leukotriene, D4 has been shown to be 1000 times more potent than histamine in terms of inducing airway obstruction[280] and nasal responses[281]. Further evidence that leukotrienes are involved in acute asthma is seen by the presence of high levels of urinary leukotriene E4 after an exacerbation[282], and by the fact that the early and late asthmatic response can be significantly attenuated with blockade by a leukotriene antagonist and antihistamine[283]. Nakamura et al[284] have shown a reduced inflammatory cellular infiltrate in a biopsy study and Pizzichini et al[285] have shown reduced eosinophil counts in the induced sputum of asthmatic patients receiving leukotriene receptor antagonists.

The development of the 5-lipoxygenase inhibitor (zileuton) and antagonists of the cysteinyl leukotriene receptor (montelukast, zafirlukast, and pranlukast) have therefore been used in the management of asthma[259]. All of the available drugs modifying the leukotriene pathway are licensed in tablet form. This obviates any problems with inhaler technique and lung delivery, although with zafirlukast there is a decrease in bioavailability when taken with food. Zafirlukast also inhibits cytochrome P450 hepatic microsomal enzymes at clinically therapeutic doses which may result in drug-interactions[286]. However, both zafirlukast and montelukast appear to be generally well tolerated and most side effects are mild e.g. gastrointestinal disturbance, rashes and fatigue[287].

Several mulitcentre studies have shown leukotriene receptor antagonists to improve clinical efficacy when compared to placebo. Altman et al[288] performed a dose-ranging study with montelukast at doses of 10mg, 100mg and 200mg given once daily; and 10mg and 50mg twice daily. There was significant improvement with all doses compared to placebo but no dose-response effect or relationship to dosing interval. Reiss et al[289] reported significant improvement in terms of spirometry, symptoms, reliever therapy usage and exacerbation rates with montelukast in patients with moderately severe chronic asthma. Interestingly the effects were evident after therapy for 1 day, and there was no evidence of tolerance. Similar results with montelukast in children and adults have also been reported[290-292]. Other authors have shown clinical efficacy with zileuton, zafirlukast and pranlukast[293-295].

There are only a few trials which have compared leukotriene receptor antagonist with other forms of asthma therapy. A comparison between inhaled sodium cromoglycate and zafirlukast[296], showed both drugs to have better control of asthmatic symptoms than placebo. However, there was no difference between the active treatments. In a recent study, Malmstom et al[297] compared 200μg twice daily beclomethasone dipropionate and 10mg once daily montelukast in patients with moderately severe chronic asthma. Both drugs were shown to have beneficial clinical effects compared to placebo in terms of spirometry, peak flow, exacerbation rates and quality of life. However, beclomethasone dipropionate was significantly better than montelukast. The only other published data shows beclomethasone dipropionate to be more effective than zafirlukast[298]. This suggests that leukotriene antagonists may not be as potent as anti-inflammatory drugs, although there is evidence to suggest they have bronchodilator properties[299].

Although the role of leukotriene receptor antagonists is not established, they are likely to have their greatest use as second-line therapy in asthmatic patients receiving inhaled corticosteroids, as inhaled corticosteroids seem to be more potent at controlling inflammation. However, there are few published reports comparing leukotriene receptor antagonists and other second-line therapy in the one study. The study by Busse et al[300] showed salmeterol to be more effective than zafirlukast in terms of pulmonary function and symptom control, whereas Turpin et al[301] showed that montelukast was superior to

inhaled salmeterol in the prevention of exercise-induced bronchoconstriction. In a multi-centre trial of patients with persistent asthma, 80% of whom were receiving concomitant inhaled corticosteroids, treatment with salmeterol produced significantly greater improvements than zafirlukast in overall asthma control, as assessed by peak flow, asthma symptoms and rescue β_2 inhaler usage[300]. This study, however, did not evaluate the effects on bronchial hyperresponsiveness (e.g. methacholine challenge) or airway inflammation.

There may be some cases for using leukotriene receptor antagonist as first-line agents. For example patients who have aspirin sensitive asthma have been shown to have over expression of leukotriene C4 synthase[302], and would benefit from these drugs[303]. It is also recognised that after exercise there is an increase in excretion of leukotrienes in the urine and this is attenuated by montelukast[304]. Other studies have shown beneficial effects of leukotriene receptor antagonists at controlling exercise induced asthma in adults[292,304,305] and children[306]. Indeed, Bronsky et al[307] showed that the montelukast caused dose-related protection against exercise-induced bronchoconstriction. Villaran et al[308] compared the efficacy of montelukast and salmeterol in controlling exercise induced asthma in 333 asthmatic patients, 25% of whom were taking low dose inhaled corticosteroids, in terms of fall in FEV_1 and showed the leukotriene receptor antagonist to be more effective after 8 weeks of therapy. Many patients do not like using inhalers and prefer the convenience of taking medication in oral form. For these patients and others with poor compliance, a once daily oral leukotriene receptor antagonist would be

an effective first line therapy.

Given that asthma and allergic rhinitis both have similar pathophysiologies, it is likely that leukotriene receptor antagonists may be effective in both conditions. Again there are little data on this area of therapeutic intervention. Knapp et al[309] showed that 5-lipoxygenase inhibition reduced allergen induced nasal congestion and levels of leukotrienes in nasal lavage fluid. Studies with leukotriene receptor antagonists have also shown improved symptom control compared to placebo in seasonal allergic rhinitis[310,311]. Malmstrom et al[312] showed that the addition of an antihistamine (loratadine) to a leukotriene receptor antagonist (montelukast) exhibited an additive effect to both treatments when given alone. However, there have been no studies investigating the use of leukotriene receptor antagonists in patients with both allergic rhinitis and asthma.

1.6 AIMS

The aim of this thesis is to perform a series of pilot studies to investigate aspects of anti-inflammatory medication used to treat allergic airways disease. Comparisons of corticosteroids given by the inhaled or intra-nasal route are made using sensitive measures of systemic activity. These include basal and dynamic tests of HPA-axis activity and markers of bone metabolism. The dose-response effects of measures of asthmatic control and airway inflammation are also assessed for inhaled budesonide. The effects of long-acting β_2 agonists and leukotriene receptor antagonist are evaluated

in comparison to inhaled corticosteroids as mono-therapy. Finally these drugs are compared on a "head-to-head" basis as second-line therapy in patients not controlled on inhaled corticosteroids.

CHAPTER 2

METHODS

2.1 SUBJECTS

Patients with asthma and seasonal allergic rhinitis were recruited from respiratory outpatient clinics at King's Cross Hospital, the Rhinology Clinic at Ninewells Hospital, Dundee, and by advertisement within Ninewells Hospital and the local press. Healthy subjects were recruited from the staff and students of the University of Dundee. All patients filled in a questionnaire giving details of past and present illnesses, history of illness in family members, and any potential risk of blood borne infections. Prior to recruitment into a study, all had a full physical examination and normal urinalysis; haematological and biochemical profile, and were negative to hepatitis serology markers. Female subjects of childbearing age were asked to provide a morning urine sample for a human chorinoic gonoadotropin pregnancy test. All asthmatic patients were non-smokers and had asthma according to American Thoracic Society criteria[202]. Patients with seasonal allergic rhinitis conformed to international criteria[14]. No subject had had an exacerbation of asthma or rhinitis which required the use of antibiotics or oral corticosteroids within 6 months prior to a study. None of the healthy volunteers were receiving any regular medication.

2.2 ETHICAL APPROVAL

Ethical approval was granted for all studies by the Tayside Committee on Medical Research Ethics. All subjects gave their written informed consent. The patients' and healthy volunteers' General Practitioners were informed and invited to discuss their views on each subjects' inclusion into a particular study.

2.3 MASKING

All inhalers, nasal sprays, tablet bottles and nebuliser solutions were masked and sealed in envelopes by a pharmacist along with instruction sheets at the beginning of each trial, in order to blind the investigators and subjects to the study medication. Where possible, an identical placebo was used. However, if this was not available all clues to the identity of the medication and dose were removed as far as possible. For many of the investigations, the subjects would not have seen the inhaler devices prior to the study, i.e. for the non UK medication such as triamcinolone acetonide, flunisolide and the Flovent formulation of fluticasone propionate. Likewise no patients would have seen the American preparation of nasal fluticasone propionate (Flonase) or triamcinolone acetonide (Nasacort) as these studies were performed prior to licensing in the UK. Identical placebo Turbuhalers and Accuhalers were available. All studies using Pulmicort Turbuhaler and Flixotide Accuhaler were, therefore, double blind. If an identical placebo was not available a similar devices was used for placebo. For studies which involved two different forms of medication e.g. inhalers and tablets, or inhalers and nasal sprays, a corresponding placebo device was given during the active treatment arm in order to make the study "double-dummy". For all studies, a randomisation code was produced and kept by a third party observer.

2.4 INSTRUCTIONS

In each study the medications were given according to the manufacturers' packet insert instructions. Prior to each study and at each visit, subjects were given detailed tuition, by

a third party, in how to use their inhalers or nasal sprays. Nasal sprays were primed according to the manufacturers' instructions prior to first use and discharged twice before each treatment was administered. After each dose of inhaled corticosteroid, all patients were instructed to rinse their mouth three times. In each study using a pressurised metered dose inhaler, a Vitalograph aerosol inhalation monitor device (Vitalograph, Bucks, UK) was used to check the subjects' co-ordination between inspiration and actuation. Likewise, in each study using a Turbuhaler an appropriate training device was used to ensure adequate peak inspiratory flow rate (Astra Draco, Lund, Sweden). In every study, each subject received a detailed written instruction sheet to follow while taking their inhaler at home and a simple tick chart was used as an aid to compliance. Patients were required to exhibit at least 90% compliance in order for the data to be considered evaluable.

2.5 SERUM/PLASMA CORTISOL

2.5.1 Basal measures

Patients attended the laboratory at least 30 minutes prior to the time of sampling. An intravenous indwelling cannula was inserted into the ante-cubital vein and patients were asked to rest, lying supine, for 30 minutes. After withdrawing dead space volume of 3 ml, a 5 ml blood sample was taken for cortisol. At the request of individual volunteers, topical anaesthetic cream (lignocaine gel) was applied to the arm 2 hours prior to venepuncture. For serum samples, blood was allowed to clot at body temperature for 20 minutes. Serum and plasma samples were centrifuged at 4°C at 3300 rpm for 15

minutes. The supernatant (serum or plasma) was aliquoted and stored at –20°C until analysed in batches, in duplicate, at the end of each study.

Analysis of serum/plasma cortisol was made by a commercially available radio-immunoassay (RIA). For the first two studies in Chapter 3 and the last in Chapter 5 an Immunodiagnostic (Immunodiagnostic System Ltd, Boldon, Tyne & Wear UK) RIA kit was used. For all other studies an Incstar (Incstar Ltd, Wokingham, Berkshire) RIA kit was used. The Immunodiagnostic RIA kit had a within assay co-efficient of variation (CV) of 7.1 and a between assay CV of 7.2 whereas the Incstar RIA kit had a within assay CV of 4.3% and a between assay CV of 7.2%. The CV's were calculated from the results of the studies performed in this thesis. Neither RIA had cross-reactivity with any inhaled corticosteroid although they had an 11% cross-reactivity for oral prednisolone (Chapter 4). A value of 150 nmol/l (5.4mg/dl) was taken to be the lower limit of normality for baseline serum or plasma cortisol.

2.5.2 Dynamic Stimulation Testing

2.5.2.1 Low Dose ACTH Stimulation Test

Adrenocorticotrophic hormone (ACTH) (Synacthen, Ciba Laboratories, Horsham, UK) was diluted to 0.5μg per ml by injecting the 250μg vial into a 500ml bag of 0.9% saline solution. After mixing, 1 ml aliquots were withdrawn from the bag and used for injection. Subjects received the injection immediately after a 0800 hr plasma/serum sample. Two further samples for cortisol were taken after 20 minutes and 30 minutes

respectively, to evaluate the peak cortisol response. A normal result was taken to be a post stimulation serum or plasma cortisol value greater or equal to 500 nmol/l (18μg/dl).

It was not possible to evaluate an ACTH stimulation response in any study involving patients with asthma or allergic rhinitis as it is contraindicated on the UK data sheet (Synacthen, CIBA Laboratories) for use in asthmatic or atopic subjects because of potential anaphylactic reactions. Indeed volunteers were screened for atopy with skin prick testing prior to recruitment into studies using ACTH stimulation testing. Any volunteer with Grade 1 reaction to skin testing with house dust mite, grass or tree pollen was excluded.

2.5.2.2 Human Corticotropin Releasing Factor

A 100μg bolus dose of human corticotropin releasing factor (hCRF) (Clinalfa AG, Läufelfingen, Switzerland) was given immediately after a 0800hr plasma/serum sample. Further samples for cortisol analysis were taken 30 and 60 minutes following the injection. The peak cortisol response was used for the purpose of analysis for post hCRF cortisol response. A normal result was taken to be a post stimulation serum or plasma cortisol value greater or equal to 500 nmol/l (18μg/dl).

2.6 URINARY CORTISOL.

2.6.1 10 Hour Overnight Urinary Cortisol

This sample consists of the total amount of urinary cortisol excreted between 2200hrs

and 0800 hrs the following day. Patients emptied their bladder, as normal, immediately prior to commencing the sample, and collected all voided urine in the container provided. Patients were asked to empty their bladder into the container at the end of the period to finish the collection. A normal result was taken to be a value greater or equal to 10nmol/10hr (3.6μg/10hr).

2.6.2 24 Hour and Fractionated Urinary Cortisol

In the last two studies in Chapter 5, twenty-four hour collections of urine were obtained. A daytime 12 hour collection (from 0800 hrs to 2000hrs) and nighttime (from 2000hrs to 0800hrs the following day) were obtained in a similar manner as described above (section 2.6.1). The 24 hour urinary cortisol measurement was calculated by the sum of the two twelve hour samples. A normal result was taken to be a value greater or equal to 40nmol/24hr (14.4μg/24hr). An 8am spot collection was obtained by patients voiding a sample at 0800hrs on request after drinking an adequate amount of water after rising in the morning. This sample was included when calculating the daytime and 24 hour samples. A normal result was taken to be a value greater or equal to 20nmol (7.2μg).

The volume of each collection was measured and duplicate aliquots were obtained and stored at –20°C until analysis in batches at the end of each study. The urinary cortisol was extracted from urine using dichloromethane prior to analysis. Urinary cortisol was measured using a commercial RIA kit which has no cross reactivity for any inhaled corticosteroid. For the first two studies in Chapters 3 and the last in Chapter 5, an

Immunodiagnostic (Immunodiagnostic System Ltd, Boldon, Tyne & Wear UK) RIA kit was used. For all other studies (last in Chapter 3, first two in Chapter 5, Chapters 4, 7 and 9) an Incstar (Incstar Ltd, Wokingham, Berkshire) RIA kit was used. The Immunodiagnostic RIA kit had a within assay CV of 10% and a between assay CV of 7.2%, whereas the Incstar RIA kit had a within assay CV of 6.7% and a between assay CV of 8.2%. The CV's were calculated from the results of the studies performed in this thesis.

2.6.3 Urinary cortisol/creatinine ratio

Urinary cortisol was corrected for creatinine excretion in all studies by calculating a cortisol/creatinine ratio. This obviated any errors in volume measurement and is thought to be more sensitive than an uncorrected value. However, as the excretion rates of both cortisol and creatinine are not constant it does not compensate for incomplete collections. Urinary creatinine was measured on a Cobas-Bio autoanalyser (Roche Products Ltd, Welwyn Garden City, UK). The within assay CV was 3.9% and the between assay CV was 0.63%.

2.7 OSTEOCALCIN

Blood was taken at 0800hrs (at the same time as the sample for cortisol) for osteocalcin and allowed to clot at room temperature for 60 minutes, centrifuged at 3300 rpm and stored at –20°C until analysis by radioiummoassay (Incstar Ltd, Wokingham, Berkshire, UK). The within assay CV was 3.3%.

2.8 EOSINOPHILIC CATIONIC PROTEIN

The samples of blood for ECP were collected in Vacutainer Hemogard SST silica gel containing tube (Becton Dickinson Vacutainer systems Europe, France). After collection, the samples were kept at room temperature for 60 minutes before being centrifuged at 3300 rpm for 15 minutes. The serum was collected in a separate aliquot bottle and frozen at -20°C until analysis at the end of the study. The ECP was measured using a radioimmunoassay kit (Pharmacia and Upjohn Diagnostics AB, Uppsala Sweden).

2.9 BLOOD EOSINOPHIL COUNT

Blood samples for measurement of eosinophil count were collected in ETDA containing tubes. They were analysed using an automated haematology analyser (SE-9000 Haematology analyser, Sysmex UK Ltd, Bucks, UK).

2.10 SPIROMETRY

The forced expiratory volume in one second (FEV_1), forced vital capacity (FVC) and the forced expiratory flow between 25% and 75% of forced vital capacity or forced mid expiratory flow rate (FEF_{25-75}) were performed according to criteria of the American Thoracic Society[66] using a Vitalograph compact spirometer (Vitalograph Ltd, Buckinghamshire UK) with a pneumotachograph head and pressure transducer and on-line computer assisted determination of FEV_1 and FEF_{25-75}. Forced expiratory

manoeuvers were performed from total lung capacity to residual volume. The best test FEV_1 value was taken from three consistent measurements (a coefficient of variation of less than 5% was considered acceptable). The pneumotachograph head was calibrated daily using a precision syringe (Vitalograph Ltd Buckinghamshire, UK).

2.11 TOTAL BODY PLESTHMOGRAPHY

2.11.1 Spirometry

A PK Morgan plethysmograph (PK Morgan, Kent, UK) with a pneumotachograph head and pressure transducer was used to measure forced expiratory flow volume curves (from total lung capacity to residual volume). Forced expiratory flow rates between 50% and 75% (FEF_{2575}) of forced vital capacity were determined by on line computerised analysis. Patients were seated and wearing nose clips during the tests. The best of three consecutive measurements were taken. The pneumotachograph head was calibrated daily using a 5 litre precision syringe (PK Morgan, Kent, UK).

2.11.2 Airways Resistance

Airways resistance was measured in a constant-volume pressure-compensated whole body plethysmograph (PK Morgan, Gillingham, Kent, UK) with subjects using nose-clips panting at 2 Hz and a peak-to-peak flow of 2-3 l/sec. Airway resistance and specific airways conductance was automatically calculated by an on-line computerised analysis. The mouth pressure, box pressure and pneumotachograph head were calibrated daily.

2.12 BRONCHIAL CHALLENGE TESTING

2.12.1 Methacholine Challenge Test

A microprocessor controlled dosimeter was used to deliver 10μl of aerosol of methacholine from 10 separate System 22 Turbo jet nebulisers (Medic Aid, Pagham, West Sussex, UK), during an individually timed activation of approximately two seconds at a driving pressure of 20 psi (138 kPa). Aerosol release into a mouthpiece was activated by a pressure transducer during inspiration from functional residual capacity to inspiratory capacity. Five inhalations (total of 50 μl) were taken for each incremental dose and nose clip was used in all cases.

Airway narrowing was assessed by measurement of FEV_1 with a Morgan Spiroflow pneumotachometer (PK Morgan Ltd., Gillingham, Kent, UK) with an Apple Macplus PC running software by Collingwood Measurement Ltd. (Packington, Leicestershire, UK). At each time point (5 minute intervals), the mean of the highest three of six FEV_1 measurements was calculated. In order to avoid the discomfort of completing a FVC manoeuvre each forced expiration was terminated after 1 second as indicated by an audible signal from the computer. Baseline FEV_1 was taken to be the overall mean from the 3 consecutive time points prior to methacholine administration.

Doubling concentrations of methacholine from 0.0625 to 32 mg/ml (doubling cumulative doses of 3.125 to 3200μg) were administered at five minute intervals until a

fall in FEV_1 greater than or equal to 20% was recorded. The methacholine provocation dose required to cause exactly 20% fall in FEV_1 (PD_{20}) was calculated by computerised interpolation of the steep part of the log dose-response curve for methacholine and FEV_1. If the FEV_1 did not show a 20% drop when a cumulative dose of 3200 µg had been inhaled, a censored PD_{20} value of 6400µg (double the maximum cumulative dose) was used for that test for the purpose of statistical analysis.

2.12.2 Adenosine Monophosphate Bronchial Challenge

Fresh solutions of adenosine monophosphate (AMP) in a range of concentrations from 0.04mg/ml to 800mg/ml were made up in normal saline on each day of the study. A nebicheck nebuliser controller (PK Morgan Ltd., Rainham, Kent, UK) was used with a system 22 Acorn nebuliser (Medic Aid Ltd., Pagham ,West Sussex, UK) with a driving pressure of 20 psi (138KPa). The nebuliser was activated for 1.2 seconds from the initiation of respiration. A mouthpiece was used with the nebuliser and the nose clipped during the procedure. The mouthpiece was placed between the teeth of the subject who exhaled to slightly below functional residual capacity and then inhaled slowly over 1 to 2 seconds toward total lung capacity, where the breath was held for 3 seconds before taking the next breath.

Baseline pulmonary function was assessed by measurement of FEV_1 using a Vitalograph compact spirometer (Vitalograph Ltd., Buckinghamshire UK) as described above (see section 2.10). Subjects then inhaled five breaths of a normal saline control solution

followed by sequential doubling concentrations of AMP given at 3 minute intervals. FEV_1 was measured 1 minute after administrating saline and each concentration of AMP. The test was terminated when a 20% fall in FEV_1 from the post-saline value was attained. The PC_{20} was calculated using a computer assisted curve fitting package (Biolab Assistant 1.1, University of Dundee, UK) and interpolation of the steep part of the log dose-response curve. A value of 1600mg/ml (double the maximum) was assigned if the FEV_1 did not fall below 20% of baseline value.

2.13 NITRIC OXIDE

2.13.1 Exhaled Nitric Oxide

Exhaled nitric oxide was measured using a chemiluminescence analyser (Model LR2000; Logan Research, Rochester, UK) sensitive to NO from 2 to 5000ppb, with a resolution of 0.3 ppb, and a response time of 2 seconds, which was designed for on-line recording of exhaled NO concentration. The NO analyser also measured CO_2, (resolution 0.1%, response time 200ms) and sample pressure and volume in real-time. The sampling rate for the analyser was 250ml/min and the analyser was calibrated weekly using a cylinder of nitric oxide at concentration of 108ppb (BOC special gases, Surrey research park).

Patients performed the test in the standing position without wearing nose clips. Measurements of exhaled NO were made by exhalation from total lung capacity for 20-30 seconds via a wide bore inert (Teflon) tube into the analyser with a flow of 15 l/min

creating a mouth pressure of 70 mmH_20 in order to cause elevation of the soft palate so that the nasal cavities are partitioned from the remainder of the respiratory tract preventing contamination of exhaled air. The mouth pressure was kept constant as patients maintained a constant expiratory flow rate using a visual feedback system with a light emitting diode visual display of expiratory flow measured by pressure and volume sensors in the analyser. The conditions of temperature (20°C), flow rate (250ml/min) and mouth pressure (70 mm H_20) were standardised throughout the study.

The initial peak (corresponding to dead-space and nasal contaminated air) was ignored and the value was taken at the plateau at the last part of exhalation. This plateau value, corresponding to the plateau of end-exhaled CO_2, has been shown to represent lower airways and alveolar sampling by direct sampling via a bronchoscope[313]. Three measures of nitric oxide were taken after intervals of at least one minute and the results were recorded.

2.13.2 Nasal Nitric Oxide

Nasal nitric oxide (NO) was determined using the same chemiluminescence analyser (Model LR2000, Logan Research, Rochester, UK). Nasal NO was measured during breath-holding as assessed by simultaneous measurement of exhaled carbon dioxide as this method keeps the soft palate closed[314]. Patients inserted the Teflon tubing in one nostril and ambient room air (NO free) was drawn up one nostril, exiting from the other, into the analyser. The analyser generated the negative pressure and kept a constant flow

rate of 250ml/min. The value was taken as the plateau value of NO. A test was deemed unacceptable if there was a rise in exhaled CO_2. Three measures of nitric oxide were recorded.

2.14 DIARY CARD

2.14.1.1 Peak Expiratory Flow Rate

Peak expiratory flow (PEF) was measured using a portable Mini-Wright peak flow meter (Clement Clark International Ltd., Harlow, Essex UK). Domiciliary measurements were made twice daily at approximately the same time each day (+/- 1 hour) at 8am and 8pm. Patients withheld their inhaled short-acting β_2 agonist reliever therapy for 6 hours prior to measurement. The test was performed, while standing, by exhaling forcefully on three occasions and recording the highest value. For a given study, each patient used the same individual peak flow meter.

2.14.1.2 Nasal Peak Inspiratory Flow Rate

Nasal peak inspiratory flow rate (nPIFR) was measured using a portable inspiratory flow meter (In-check Clement Clarke International Limited, Harlow, Essex, UK) with a purpose built facemask. Prior to testing, patients were asked to blow their nose to expel secretions. All measurements were made in the sitting position, with a good seal round the facemask and patients inspired forcefully from residual volume to total lung capacity through their nose, with the mouth closed according to manufacturer's packet insert instructions. Measurements were made twice daily, at approximately the same time each

day +/- 1-hour, at 8am and 8pm, throughout the study. Three maximal inspiratory efforts were performed, the highest value being recorded by the patient. For a given study, each patient used the same individual inspiratory flow meter.

2.14.2.1 Asthma Symptoms

Asthma symptom scores were recorded according to a four-point scale, with zero indicating no symptoms and three indicating maximal symptoms, twice daily at 8am and 8pm. Patients recalled the extent of their symptoms over the preceding 12 hours.

2.14.2.2 Seasonal Allergic Rhinitis Symptoms.

Seasonal allergic rhinitis symptoms were recorded under four headings, namely nasal, eye and throat symptoms, and the effect of their condition on their daily activity. Symptom scores were documented according to a 4-point scale with 0 representing no symptoms and 3 representing maximal symptoms. Under the heading of nasal symptoms, patients recorded "runny nose", "blocked/stuffy nose", "itchy nose" and "sneezing". For eye symptoms, patients recorded "itchy eyes", "watery eyes" and "red eyes". Under the heading of throat symptoms, patients recorded "tickly throat". Patients were also asked to record the extent to which their symptoms interfered with their daily activity on an 11-point scale, with 0 representing no symptoms and 10 representing maximal symptoms.

2.14.3 Rescue Therapy Usage

On a twice daily basis, at 0800hrs and 2000hrs, patients recorded the number of puffs of rescue medication with inhaled reliever therapy (short-acting β_2 agonist or anticholinergic inhalers) that they had taken during the preceding 12 hours. Patients also recorded their requirement for use of ocular sodium cromoglycate eye drops in number of drops used during the preceding 24 hours at 0800hrs.

2.15 ALLERGY TESTING

2.15.1 Skin Prick Testing

Patients withheld anti-histamine medication for 4 days prior to skin prick testing. This was performed following a standard protocol (Bencard testing solutions, Welwyn Garden City, UK) using extracts including grass, tree and weed pollen in addition to a negative control. Results were read after 10 minutes, a positive reaction being defined as a minimum weal diameter with erythema of 2mm greater than negative control.

2.15.2 Phadiotop Testing

Patients were assessed for the presence of atopy by multiple allergen testing of their serum to a battery of inhaled allergens including mites, molds, trees, grasses weeds, cats and dogs by radioallergosorbent testing (Uni Cap Phadiotop test, Pharmacia Upjohn Ltd., Milton Keynes, UK).

2.16 POLLEN COUNT MEASUREMENT

Data were collected locally on a daily basis, (Scottish Crop Research Institute, Dundee,

UK) using a 7 day recording volumetric spore trap (Burkard Manufacturing Co Ltd., Hertfordshire, UK).

2.17 STATISTICAL ANALYSIS

Each study was powered, where possible, as detailed. Data were entered into a computer and checked in duplicate. All bronchial challenge data were log transformed as were other data if they did not conform to a normal distribution. For bronchial challenge testing, data from active treatment were compared to placebo or baseline in terms of the fold difference. This comparison is commonly expressed as the number of "doubling dose differences" from placebo. This is calculated as the log of the fold difference divided by the $\log_{10}$ of 2 (0.30103).

For all parameters (active treatments at each dose and placebos) comparisons were made by an overall multifactorial analysis of variance (MANOVA) using treatment, dose, subject and period as factors. Where a significant overall difference between treatments and placebo was found multiple range testing was applied to identify where these differences occurred. A value of $p<0.05$ (two tailed) was considered to be significant and 95% confidence intervals for mean treatment differences or fold differences were calculated.

The presence of dose related suppression was determined using least squares regression analysis to evaluate the overall effects of all doses of each drug studied, where

appropriate. In Chapter 4, parallel slope analysis was then used where possible and in the presence of a significant fit for the common parallel slope a dose ratio was calculated for relative potency.

The Area Under the Curve (AUC) plasma cortisol was generated from the integrated 24 hour plasma sample profile using the trapezoidal method and the fractionated (overnight, 8am, daytime) components were also analysed separately. The fractionated (overnight, 8 am, daytime) and 24 hour urinary cortisol collections were analysed after correcting for urinary creatinine excretion.

The number of individual values of a measurement below the lower limit of normal was analysed using the Chi- square test.

All data were analysed using a 'Statgraphics' software package (STSC Software Group, Rockville, Maryland, USA).

2.18 MEDICATION

beclomethasone dipropionate (Vancenase AQ double strength, Schering Corporation, Kenilworth, USA)

budesonide (Pulmicort pMDI , Astra Pharmaceuticals Ltd, Kings Langley, Herts, UK)

budesonide (Pulmicort Respules, Astra Pharmaceuticals Ltd, Kings Langley, Herts, UK)

budesonide (Pulmicort Turbuhaler, Astra Pharmaceuticals Ltd, Kings Langley, Herts, UK)

budesonide (Rhinocort Aqua, Astra Pharmaceuticals Ltd, Kings Langley, Herts, UK)

flunisolide (Aerobid, Forest Pharmaceuticals Inc, St Louis, USA)

fluticasone propionate (Flixotide pMDI, Allen & Hanburys, Uxbridge, UK)

fluticasone propionate (Flonase, Glaxo Wellcome Inc, USA)

fluticasone propionate (Flovent metered dose inhaler, Glaxo-Wellcome Inc, USA)

formoterol (Oxis Turbuhaler, eformoterol fumarate, Astra Pharmaceuticals Ltd, Kings Langley, Herts, UK)

ipratropium bromide (Atrovent Forte, Boehringer Ingelheim, Bracknell, UK)

mometasone furoate (Nasonex, Schering-Plough Ltd, Hertfordshire, UK)

montelukast (Singulair, Merck Sharpe & Dohme Ltd, Herts, UK)

prednisolone (5mg tablets, Biorex Laboratories Ltd, Enfield, UK)

salbutamol (Ventolin Accuhaler, Allen & Hanburys Ltd, Uxbridge, UK)

salmeterol (Serevent Accuhaler, Allen & Hanburys Ltd, Uxbridge, UK)

sodium cromoglycate (Clariteyes, Sheering Plough, Welwyn City, UK)

triamcinolone acetonide (Azmacort oral inhaler, Rhone Poulenc Rorer Pharmaceuticals, Collegeville, USA)

triamcinolone acetonide (Nasacort AQ, Rhone Poulenc Rorer Pharmaceuticals Inc, Collegeville, USA.

CHAPTER 3

DOSE RESPONSE COMPARISON FOR RELATIVE SYSTEMIC EFFECTS OF INHALED CORTICOSTEROIDS

Study 1 A Comparison of the Systemic Effects of Inhaled Triamcinolone Acetonide and Fluticasone Propionate Adult in Asthmatic Patients

Study 2 A Comparison of the Systemic Effects of Inhaled Triamcinolone Acetonide and Flunisolide in Healthy Adult Volunteers

Study 3 A Comparison of the Systemic Effects of Inhaled Fluticasone Propionate Given By Two Devices with Different Lung Delivery

3.1 INTRODUCTION

This chapter examines dose-response comparisons for relative systemic effects of inhaled corticosteroids. There are three separate studies each of which look at different aspects of systemic activity. The first is a dose-response study comparing triamcinolone acetonide and fluticasone propionate in asthmatic patients, which assesses systemic activity in terms of basal measures of adrenal function. The second evaluates triamcinolone acetonide and flunisolide by assessing basal and dynamic adrenal function using the ACTH stimulation test. As ACTH stimulation test now contra-indicated in the UK for use in asthmatics (see section 2.5.2.1), healthy volunteers were recruited. The third study evaluates the influence of the drug device on the degree of systemic activity, again in healthy volunteers. None of the studies investigate the clinical efficacy of the drugs or the therapeutic ratio.

A pilot study[315] was performed to compare the adrenal suppression of inhaled fluticasone propionate and triamcinolone acetonide in twelve healthy volunteers. Both drugs were given via their respective pressurised metered dose inhaler (pMDI) devices at high doses within the manufacturers recommended dose range. The study was of a single (investigator) blind randomised crossover design and compared a total daily dose of 1625μg fluticasone propionate delivered via a pMDI, 1600μg daily of triamcinolone acetonide delivered via a pMDI with integrated tube spacer, or placebo pMDI. All treatments were given in two divided doses at 8.00 am and 10.00 pm over a 24 hour period. Each drug treatment was separated by a 1 week washout. Blood samples were

taken for 8.00am plasma cortisol, i.e. 10 hours following the second dose. 10 hour overnight urine collections were taken for urinary cortisol and creatinine excretion. For 8.00 am plasma cortisol (geometric mean, nmol/l) compared with placebo (353.1) fluticasone propionate (137.7) produced significant ($p<0.05$) suppression (2.57-fold difference: 95% CI 1.50 to 4.39), whereas triamcinolone acetonide (262.8) did not (1.34-fold difference: 95% CI 0.77 to 2.34). Fluticasone propionate produced 1.91-fold greater adrenal suppression ($p<0.05$) than triamcinolone acetonide (95% CI 1.10 to 3.33). Individual subjects with abnormal low 8.00 am cortisol values < 150nmol/l (< 5.4μg/dl) were n=4 for fluticasone propionate and n=0 for triamcinolone acetonide ($p<0.05$). Overnight urinary cortisol/creatinine ratio (geometric mean, nmol/mmol) did not show any difference between fluticasone propionate (1.48) and triamcinolone acetonide (1.60), with both producing significant suppression versus placebo (4.01): triamcinolone acetonide 2.50 fold difference (95% CI 1.45 to 4.24), fluticasone propionate 2.71 fold difference (95% CI 1.57 to 4.69). Therefore fluticasone propionate 1625μg per day produced approximately two-fold greater adrenal suppression of 8.00 am plasma cortisol than triamcinolone acetonide 1600μg per day when given twice daily, and one third of subjects with fluticasone had abnormal low 8.00 am cortisol values <150nmol/l. There were no differences between the drugs for urinary cortisol excretion.

However, as this was a pilot study, there were several limitations to the study that may result in the findings not being clinically relevant. Firstly, the subjects were healthy volunteers and had normal airway caliber. As the systemic activity of inhaled

corticosteroids like fluticasone propionate and triamcinolone acetonide with a high degree of first-pass metabolism is mainly determined by the lung delivery, volunteers with normal airway caliber will have greater systemic absorption. Thus the degree of adrenal suppression compared to placebo is likely to be different between volunteers and patients with airways obstruction. Furthermore there may be other differences between patients with asthma and healthy volunteers including differences in muco-ciliary clearance and attitudes towards taking medication. In short, it is only clinically relevant to evaluate the response of drugs in patients who would normally be taking them.

Secondly the measurements were made after one day's dosing and therefore the blood and tissue levels of the inhaled corticosteroids could not have reached steady state. As the degree of adrenal suppression is related to steady-state drug levels, a single day's dosing will not represent the clinical setting. In this respect Lonnebo et al[316] showed that fluticasone propionate had greater systemic activity after repeated dosing compared to after a single dose. Furthermore, as discussed above (see section 1.4.1), single dose studies offer little information regarding the relative effects of two drugs. More meaningful results are obtained from dose-response rather than single dosing studies when comparing the relative systemic bioactivity of different inhaled corticosteroids. For these reasons the study was repeated in the first study of this chapter in order to compare fluticasone propionate and triamcinolone acetonide in a dose-response manner at steady-state dosing interval in patients with asthma. The doses chosen represent low medium and high treatments and were given by their respective pressurised metered dose inhalers.

The second study assesses the effects of inhaled corticosteroids on basal and dynamic measures of HPA-axis activity. Although 8am serum cortisol and overnight urinary cortisol/creatinine ratio are recognised to be sensitive and reproducible markers of systemic bioactivity, measuring adrenal suppression in terms of early morning serum cortisol concentration or urinary cortisol excretion only gives information about the basal adrenocortical activity. It is probably more clinically meaningful to look at the effects of stress on the hypothalamic-pituitary-adrenal (HPA) axis, as this will indicate the degree of adrenal reserve. The low dose ACTH (0.5μg) test was chosen, as there is evidence that it may be more a sensitive method of detecting impaired adrenocortical reserve than the standard (250μg) ACTH test (see section 1.3.4).

As well as comparing the relative systemic effects of inhaled corticosteroids with different pharmacological properties, for example fluticasone propionate and triamcinolone acetonide, it is also important to consider the influence of the lung delivery via an inhaler device (see section 1.3.1). For example the two-fold difference between these two drugs when delivered by their respective pressurised metered dose inhalers, elicited in the pilot study[315], may be confounded by the respective device delivery. Fluticasone propionate is a particularly good drug to use when investigating the influence of the inhaler device, as the near complete first pass hepatic metabolism will minimise any confounding influence of oral bioavailability.

3.2 METHODS

Patients

Study 1:Twelve stable mild to moderate asthmatic patients (6 female) of mean age (SE): 34.3 (2.9) years mean FEV_1: 82.1 (2.0) % predicted, and FEF_{25-75} 53.6 (5.5) % predicted, completed the study. All patients were receiving less than or equal to 400μg per day of inhaled corticosteroid. (Median dose: 250μg per day, range: 100 to 400μg per day).

Study 2: Twelve healthy volunteers (3 female) mean age (SE) 24.2 (2.4) years.

Study 3: Sixteen healthy volunteers (8 female) mean age (SE) 29.3 (2.3) years.

Study Design

Study 1: Patients were randomised to receive either triamcinolone acetonide 100 μg per actuation (dose delivered to patient as Azmacort oral inhaler with integrated spacer device, Rhone Poulenc Rorer Pharmaceuticals Inc., USA) or Fluticasone propionate 110μg per actuation (dose delivered to patient as Flovent metered dose inhaler, Glaxo-Wellcome Inc., USA). Six patients received fluticasone propionate first in sequence and the other six patients received triamcinolone acetonide first in sequence. Each drug sequence was given over a total of 9 days in twice daily divided doses at 8am and 10pm. The doses were as follows given sequentially each for three days: TAA: 2 puffs bid, 4 puffs bid and 8 puffs bid (i.e. total daily dose of 400μg, 800 μg, 1600μg, respectively); FP: 2 puffs am/1 puff pm, 4 puffs am/3 puffs pm, 7 puffs bid (i.e. total daily dose of 330μg, 770μg, 1540 μg, respectively). Prior to each 9 day drug sequence (i.e. either fluticasone propionate or triamcinolone acetonide) patients received the respective

matching placebo inhaler (pressurised metered dose inhaler (pMDI) or oral inhaler) 2 puffs bid also for three days. The patients' usual inhaled corticosteroid therapy was discontinued during the placebo and treatment periods. There was also a 12 day washout between each of the 9 day treatment sequences where patients received their usual maintenance inhaled corticosteroid therapy. Each inhaler was discharged twice prior to inhalation and each inhalation was followed by mouth rinsing. Spirometry was also measured at each visit to ensure the FEV_1 did not vary by more than 15% between treatments.

Study 2: Subjects were randomised to receive either: Triamcinolone acetonide 100 μg per actuation (as Azmacort with integrated actuator/spacer, Rhone Poulenc Rorer Pharmaceuticals, Inc, Collegeville, USA) or Flunisolide 250 μg per actuation without spacer (as Aerobid, Forest Pharmaceuticals, Inc, St Louis, USA). These drugs were used according to manufacturer's labeling. Six patients received triamcinolone acetonide (TAA) first and the other six received flunisolide (FN) first in sequence. Each drug was given for six days (each dose for 3 days) in twice daily doses at 0800 h and 2200 h mouth rinsing. The dosing sequence was as follows each given sequentially for 3 days: TAA 4 puffs BID and 8 puffs BID (i.e. total daily dose of 800 μg and 1600 μg respectively); FN 2 puffs BID and 4 puffs BID (i.e. total daily dose of 1000 μg and 2000 μg respectively). The total daily dose of each drug was chosen to reflect the lowest (L) and highest (H) recommended dose according to the manufacturers' labeling. Prior to the first treatment sequence each patient received a placebo treatment sequence

2 puffs BID for three days. The placebo device used corresponded to the first treatment device (Flunisolide pMDI without spacer or Azmacort with integrated actuator/spacer). There was a 10 day washout between treatments. Each inhaler was discharged twice prior to inhalation and each inhalation was followed by mouth rinsing.

Study 3: Volunteers were randomised into a 3-way crossover study comparing placebo inhaler [PL]; 2mg of fluticasone propionate dry powder (Flixotide Accuhaler 250μg per actuation, Allen & Hanburys Ltd, Uxbridge UK) [DP]; or a pressurised metered dose inhaler (pMDI) (Flixotide 250μg per actuation) with a Volumatic spacer [pMDI+spacer]. Each treatment was given at 1800hrs, under supervision. All data were log transformed followed by multifactorial analysis of variance and Bonferroni's multiple range testing.

Measurements

Measurements were made after each dose level of both treatments and after both the run-in and washout placebo for Serum Cortisol, 10 Hour Overnight Urinary Cortisol excretion, Overnight Urinary Cortisol/Creatinine Ratio (Study 1) and for 8am Serum Cortisol, Low Dose Synacthen Test, Overnight Urinary Cortisol/Creatinine, 8am urinary cortisol/creatinine (Study 2). In Study 3, subjects attended the following day for measurement of 8am serum cortisol and collected all urine passed between 10pm on the day of dosing until 8am on the following day for analysis of cortisol and creatinine.

Statistical Analysis

All three studies were designed with sample size of 12 with 80% power (beta error =0.2) to detect a 20% difference in 8.00am cortisol (the primary end point) between treatments with the alpha error set at 0.05 (two-tailed). Comparisons between treatments were made by an overall multifactorial analysis of variance (MANOVA), followed by Duncan's multiple-range testing in the first study and Bonferroni's multiple-range test in the other two studies. In addition, a comparison was made to assess any carryover effect between the active treatment periods by comparing values for placebo in order of sequence. The presence of dose related suppression was determined using least squares regression analysis to evaluate the overall effects of all three dose levels for each drug in the first study. Data were log transformed prior to analysis so as to normalise their distribution in the first study only.

The number of individual values for overnight urinary cortisol < 3.6μg (10 nmol) and early morning urinary cortisol < 7.2μg (20 nmol); 8am serum cortisol < 5.4μg/dl (150 nmol/l) and serum cortisol response to ACTH < 18μg/dl (500 nmol/l), were analysed using the Chi-Square test in Study 2, whereas only those values for overnight urinary cortisol excretion <10nmol/10hr, at a dose less than 1000μg per day were analysed in the first study.

3.3 RESULTS

Study 1

There were no significant carryover effect between the first and second placebo using either of the parameters measured: 8am serum cortisol 574.4 vs 539.1 nmol/l and overnight corrected urinary cortisol/creatinine ratio 5.0 vs 5.3 nmol/mmol. There were no significant differences between the FEV_1 values (as % predicted) comparing placebo (PL) with low (L) medium (M), high (H) doses of each drug: PL (92.3); TAA: (L: 96.6, M: 94.9, H: 94.5); FP (L: 96.6, M: 92.3, H:95.0).

8 am Serum Cortisol:

Regression analysis showed there was significant dose-related suppression with fluticasone propionate ($p<0.001$) but not with triamcinolone acetonide [Figure 3.1]. At the highest dose there was a 2.33 fold ratio between FP and PL ($p<0.05$), and a 2.03 fold ratio between TAA and FP ($P<0.05$). There was no significant difference between TAA and PL at any dose. Compared with PL there were significant ($p<0.05$) differences with medium and high doses of FP but at no dose of TAA. Geometric means (SE) were as follows (nmol/l): PL: 574.4 (33.1); FP: L: 505.1 (28.9), M: 419.1 (41.8), H: 246.8 (44.4); TAA: L: 554.9 (32.0), M: 538.0 (53.6); H: 500.8 (90.0).

Overnight Urinary Cortisol:

Regression analysis for overnight corrected urinary cortisol/creatinine excretion showed fluticasone propionate to cause significant ($p<0.005$) dose related suppression whereas

this was not significant with triamcinolone acetonide [Figure 3.1] At the high dose there was a significant ($p<0.05$) 2.69 fold ratio between FP and PL and a 1.9 fold ratio between FP and TAA. There were significant ($p<0.05$) differences from placebo for medium and high doses of FP and for the medium dose of TAA. Geometric means (SE) were as follows (nmol/mmol): PL: 5.0 (.07); FP: L: 4.2 (0.6), M: 2.2 (0.3), H: 1.9 (0.5); TAA: L: 4.3 (0.6), 3.3 (0.4), 3.5 (1.0). For doses < 1000μg per day the number of individual results with an abnormal low value for urinary cortisol excretion (< 10 nmol/10hr) were 10/24 (42%) for FP and 3/24 (13%) for TAA ($p<0.005$) [Figure 3.2].

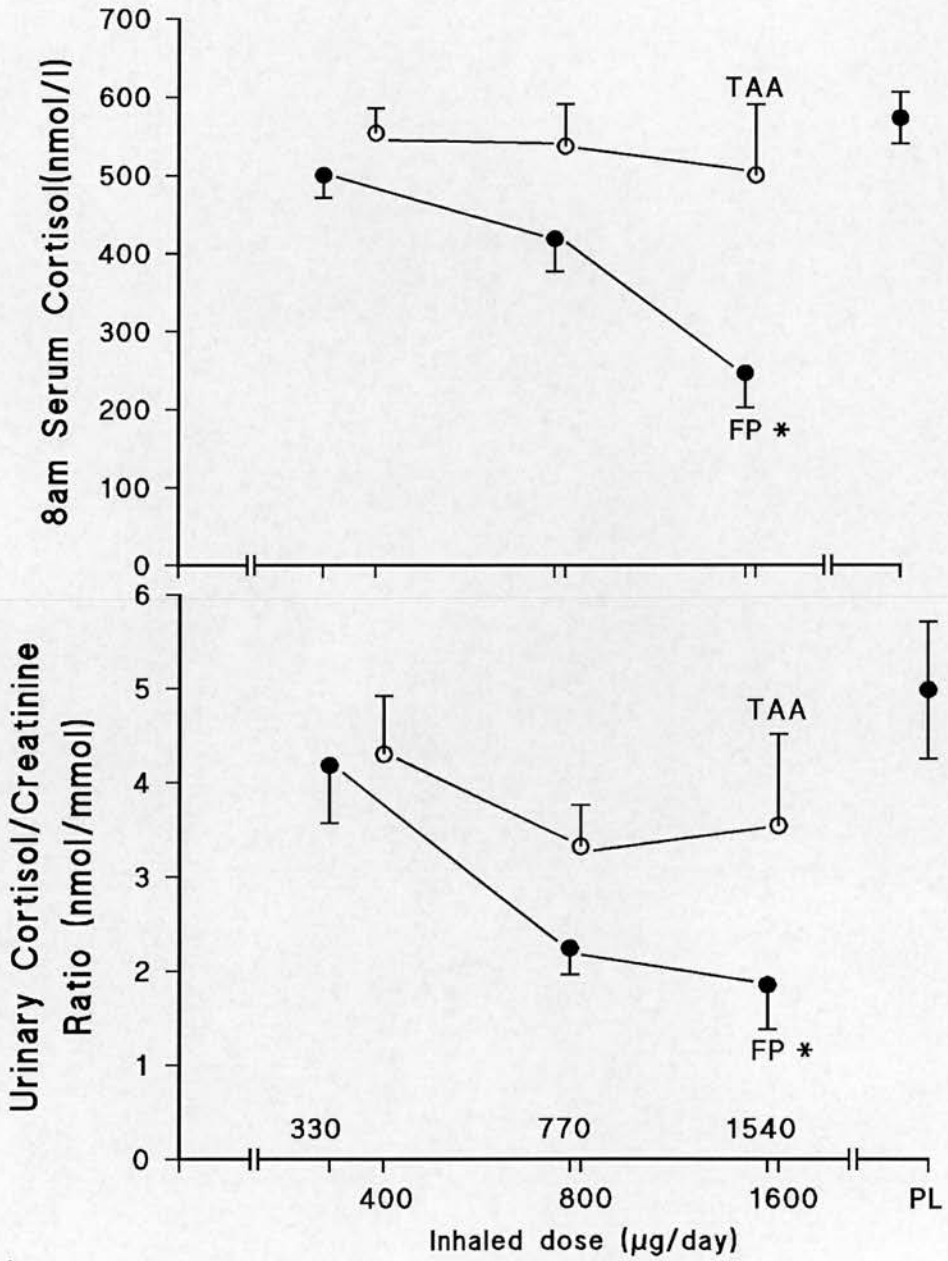


Figure 3.1

Geometric means with standard error of mean for placebo (PL); triamcinolone acetonide (TAA) at 400 µg per day, 800µg per day, and 1600µg per day; and fluticasone propionate (FP) at 330 µg per day, 770µg per day, 1540 µg per day for 8am serum cortisol (top) and overnight corrected urinary cortisol/creatinine excretion (bottom). Regression analysis showed significant dose-related suppression for fluticasone propionate (* $p<0.001$) for serum cortisol and (* $p<0.005$) for urinary cortisol/creatinine excretion, but not a significant effect for triamcinolone acetonide.

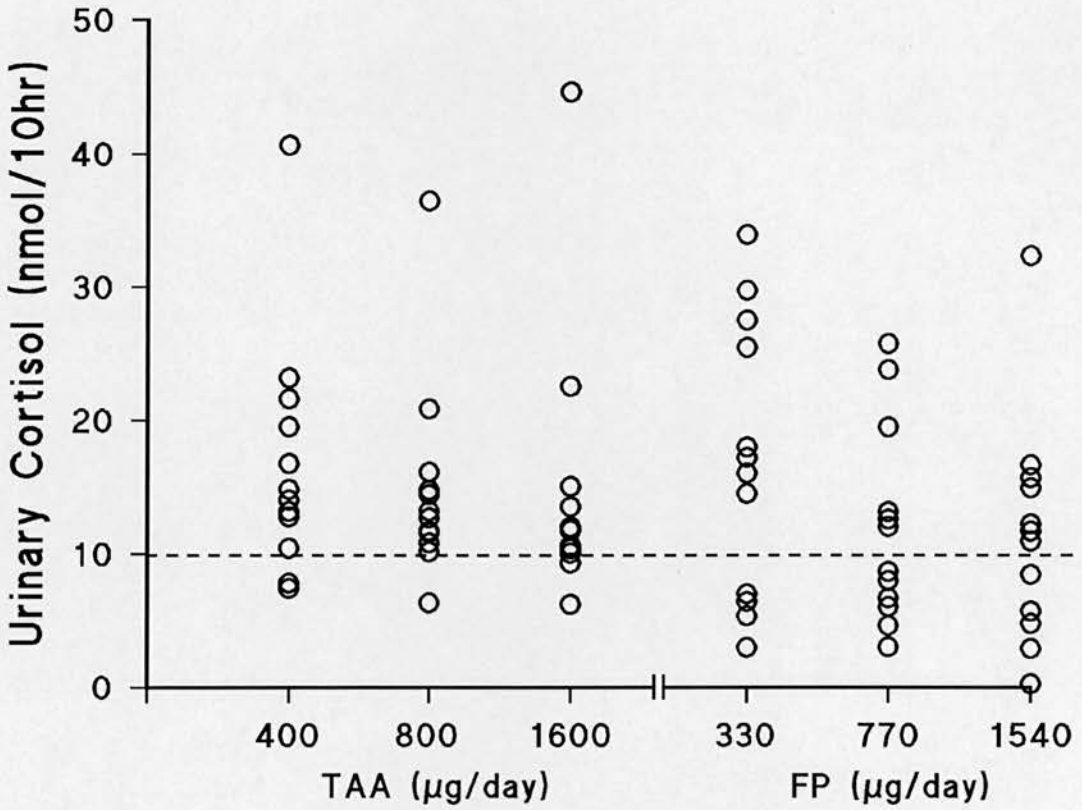


Figure 3.2

Individual values for uncorrected overnight urinary cortisol excretion for all 3 dose levels of each drug. For doses < 1000µg per day individual abnormal low levels (<10nmol/10hr) were: 10/24 (42%) for fluticasone propionate (FP) vs 3/24 (13%) for triamcinolone acetonide (TAA) ($p<0.005$).

Study 2:

There were no significant carryover effects between placebo and washout values in sequence for any of the parameters measured: a) Pre ACTH serum cortisol 481.8 vs 513.7 nmol/l, b) post-ACTH 666.3 vs 668.9 nmol/l, c) overnight corrected urinary cortisol/creatinine ratio 6.4 vs 5.7 nmol/mmol, d) 8am corrected urinary cortisol/creatinine ratio: 39.0 vs 39.5 nmol/mmol. Mean values (after placebo or washout) prior to starting treatment with either flunisolide and triamcinolone acetonide respectively were also not significantly different (FN vs TAA): a) Pre ACTH serum cortisol 502.0 vs 493.6 nmol/l, b) post ACTH 676.2 vs 659.0 nmol/l, c) overnight corrected urinary cortisol/creatinine ratio 6.3 vs 5.8 nmol/mmol, or d) 8am corrected urinary cortisol/creatinine ratio 40.9 vs 36.0 nmol/mmol.

Pre-ACTH 8 am serum cortisol:

There were no significant differences, between placebo (481.8 nmol/l) and any of the other treatments: L TAA (519.9 nmol/l), L FN (545.8 nmol/l), H TAA (388.7 nmol/l), H FN (481.4 nmol/l) [Figure 3.3]. There was 1 subject who had a value less than 5.4μg/dl (150 nmol/l) (with L FN)

Post ACTH Serum Cortisol

There was no significant difference between placebo (666.3 nmol/l) and any of the other treatments L TAA (686.0 nmol/l), L FN (699.2 nmol/l), H TAA (591.4 nmol/l) H FN (617.0 nmol/l) [Figure 3.3]. When analysing the number of individual values less than

18μg/dl (500 nmol/l) for both dose levels there was no significant difference between the drugs: 3/24 for TAA vs 2/24 for FN [Figure 3.4]. None of the post-stimulated cortisol levels were below 14.4μg/dl (400 nmol/l).

Overnight corrected urinary cortisol/creatinine excretion:

Compared with placebo (6.4 nmol/mmol) there was significant suppression ($p<0.05$) for the high dose H TAA (2.3 nmol/mmol) and H FN (2.6 nmol/mmol) but not at the low dose L TAA (4.5 nmol/mmol) or L FN (4.2 nmol/mmol). There was no significant difference between the two drugs [Figure 3.5]. When analysing individual values for both dose levels for overnight urinary cortisol less than 3.6μg (10 nmol) there were no differences between the two drugs: 13/24 for TAA vs 11/24 for FN.

Early morning corrected urinary cortisol/creatinine excretion

Compared with placebo (39.0 nmol/mmol) there was significant suppression ($p<0.05$) with the high dose of both drugs (H TAA: 26.6 nmol/mmol, H FN 26.5 nmol/mmol) but no significant suppression with the low doses (L TAA 36.5 nmol/mmol, L FN 37.2 nmol/mmol) [Figure 3.5]. When analysing values less than 7.2 μg (20nmol) for both dose levels for early morning urinary cortisol, there was no difference between the drugs: 6/24 for TAA vs 6/24 for FN.

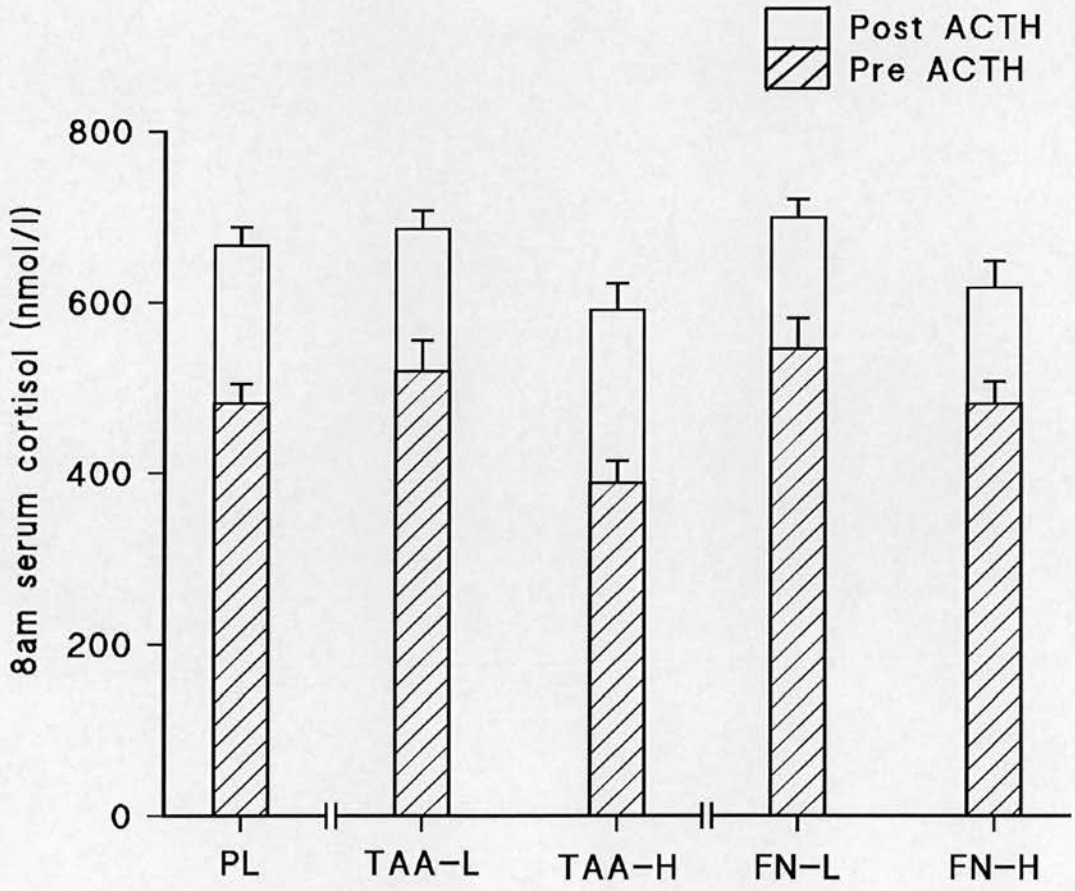


Figure 3.3
Means with standard error for placebo (PL), triamcinolone acetonide 800 μg per day (TAA-L) and 1600μg per day (TAA-H); flunisolide 1000μg per day (FN-L) and 2000μg per day (FN-H) for pre and post ACTH stimulation serum cortisol. Neither drug had any significant effect either pre or post stimulation.

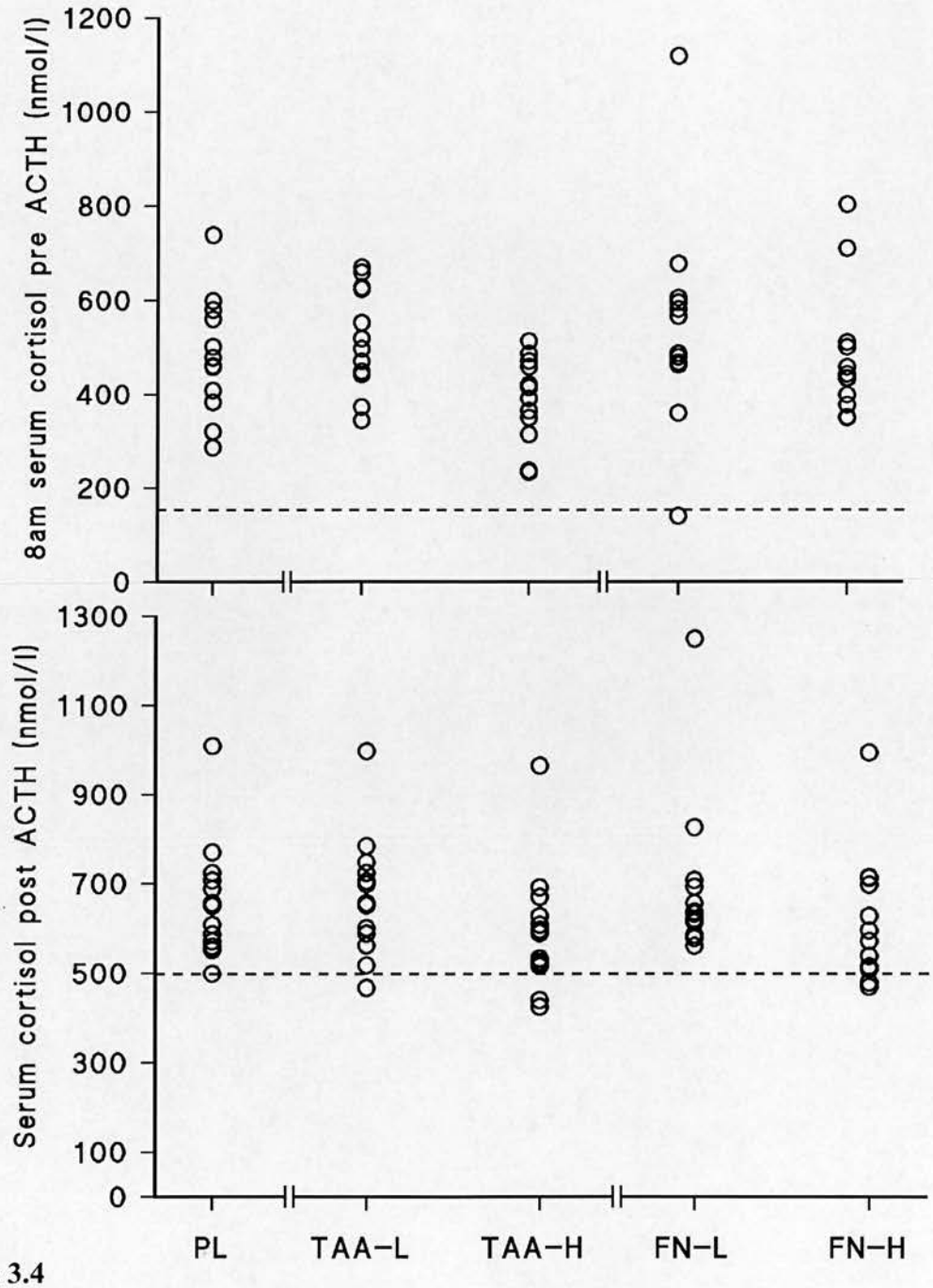


Figure 3.4

Individual values for placebo (PL), triamcinolone acetonide 800 μg per day (TAA-L) and 1600μg per day (TAA-H); flunisolide 1000μg per day (FN-L) and 2000μg per day (FN-H) for pre ACTH stimulation for (a) 8am plasma cortisol (There was only 1 abnormal low value (with FN-L) below 150nmol/l) (b) post ACTH stimulation plasma cortisol (There were 3 values for TAA and 2 for FN below 18μg/dl (500nmol/l)

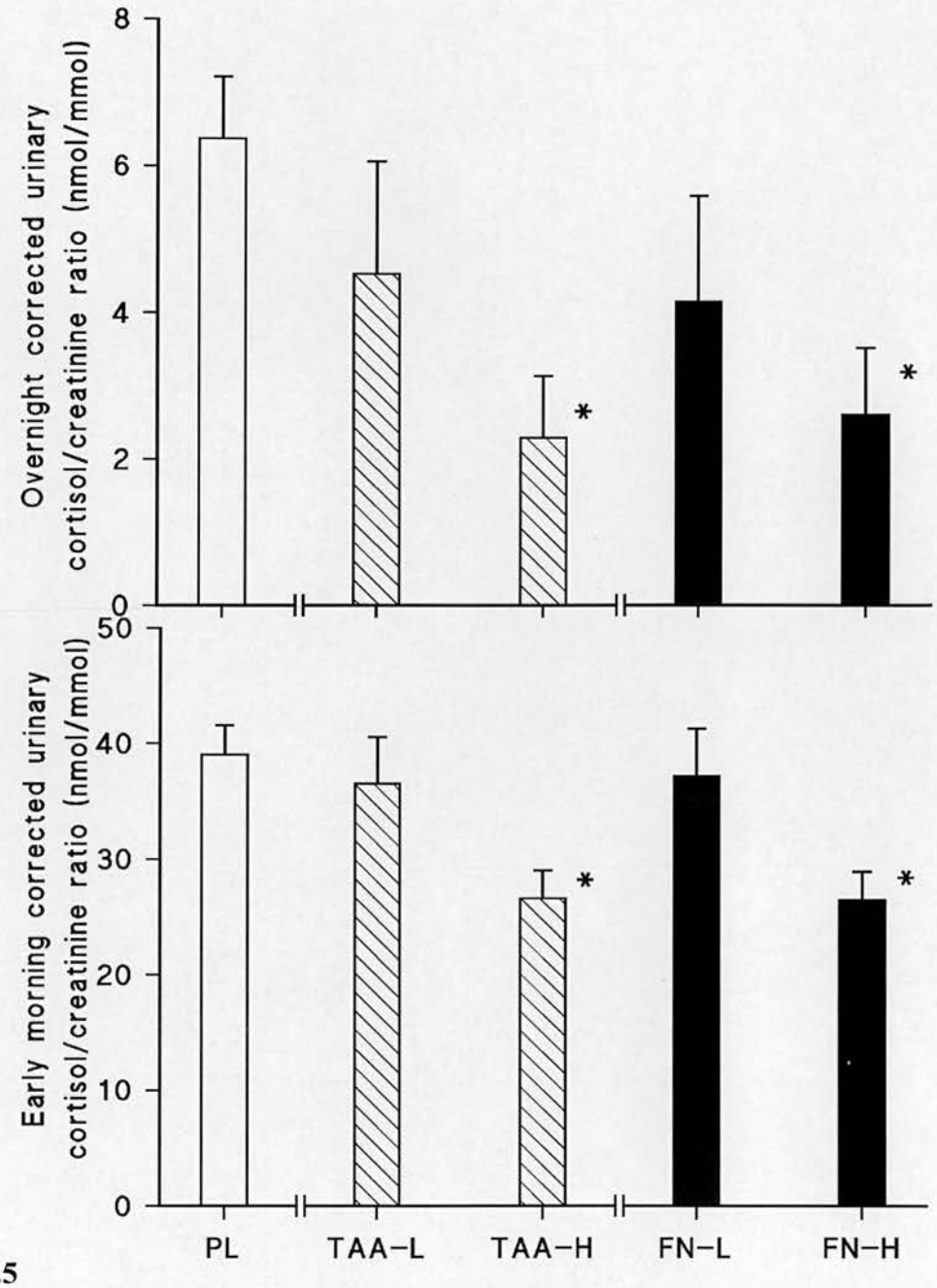


Figure 3.5

Means with standard error for placebo (PL), triamcinolone acetonide 800μg per day (TAA-L) and 1600μg per day (TAA-H); flunisolide 1000μg per day (FN-L) and 2000μg per day (FN-H) for (a) overnight and (b) early morning corrected urinary cortisol/creatinine excretion. Asterisk denotes significant ($p<0.05$) difference for either steroid from placebo.

Study 3

There were no significant differences between PL and DP for any measurement [Table 3.1]. However, there were significant differences ($p<0.05$) between pMDI+spacer versus DP [Table 3.1]. The geometric mean fold difference (95% CI for difference) between DP versus pMDI+spacer were: 5.48-fold (2.90-10.34) for urinary cortisol, 7.68-fold (4.10-14.39) for urinary cortisol/creatinine ratio, and 5.57-fold (2.97-10.43) for serum cortisol. For serum cortisol the number of subjects with an abnormal low value (<150nmol/l) were: PL n=0/16, DP n=1/16, pMDI+spacer n=10/16 ($p<0.001$ for DP vs pMDI+spacer)[Figure 3.6].

Table 3.1

Geometric means (within treatment 95% confidence intervals) for placebo inhaler (PL), 2mg nominal dose of fluticasone propionate delivered via a pressurised metered dose inhaler and spacer (pMDI+spacer), and via a dry powder Accuhaler device (DP) for 10 hour overnight urinary cortisol (OUC), overnight urinary cortisol/creatinine ratio (ONCC) and 8am serum cortisol (8am). Asterisk denotes significant ($p<0.05$) difference between active treatment and placebo, cross denotes significant difference between the two active treatments.

	PL	pMDI+spacer	DP
OUC (nmol/10hr)	41.0 (28.8 - 58.4)	4.7 *+ (3.2 - 6.8)	25.7 (18.1 - 36.6)
OUCC (nmol/mmol)	7.9 (5.6 - 11.2)	1.1 *+ (0.8 - 1.6)	8.6 (6.1 - 12.3)
8am (nmol/l)	421.6 (294.8 - 602.8)	67.2 *+ (47.0 – 96.0)	373.8 (261.5 – 534.5)

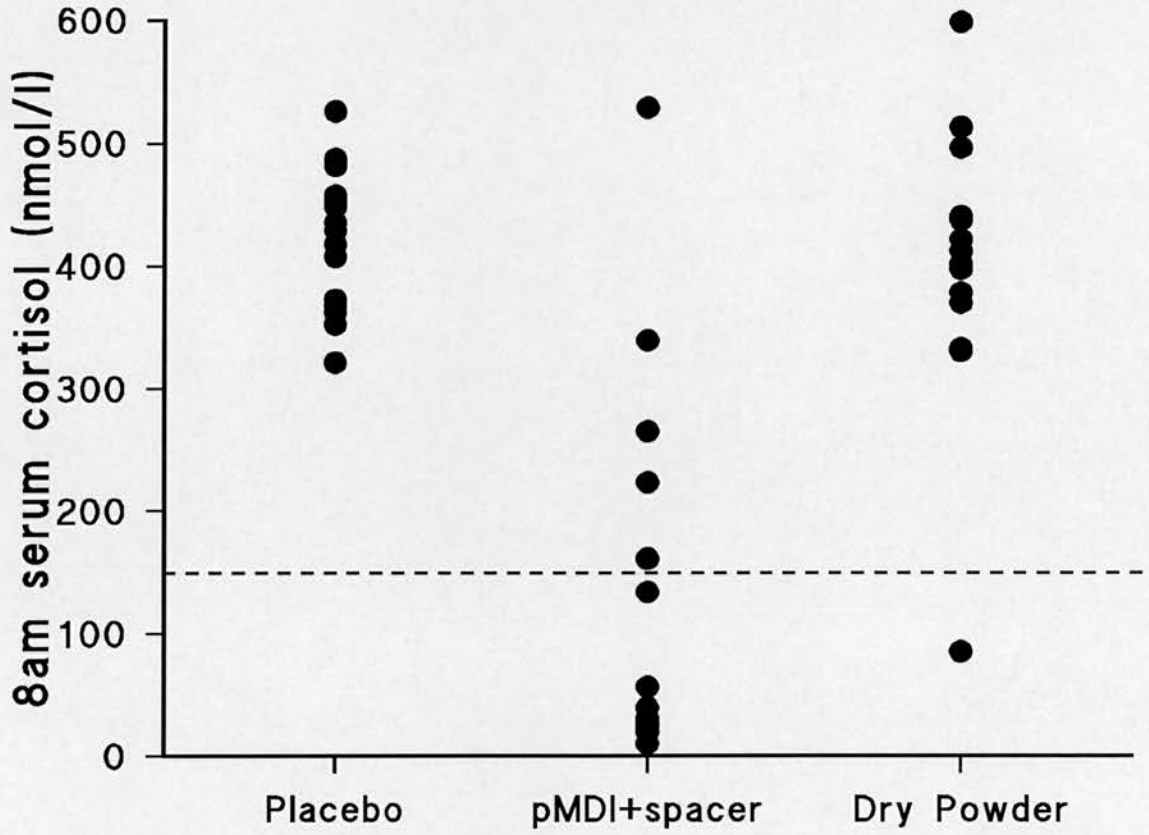


Figure 3.6
Scatter plot showing individual values for 8am serum cortisol for placebo and fluticasone propionate delivered via a metered dose inhaler and spacer (pMDI+spacer) and dry powder device. The number of subjects with an abnormal low value (<150nmol/l) were: PL n=0/16, DP n=1/16, pMDI+spacer n=10/16 ($p<0.001$ for DP vs pMDI+spacer)

3.4 DISCUSSION

In the study comparing inhaled fluticasone propionate and triamcinolone acetonide, fluticasone produced significant dose-related suppression of 8am serum cortisol and overnight corrected urinary cortisol/creatinine excretion, whereas inhaled triamcinolone did not. At the highest dose this amounted to a two fold difference between the two drugs. This is in keeping with the findings of the pilot study in normal volunteers[315] which also showed a two fold difference between these inhaled corticosteroids in terms of overnight urinary cortisol/creatinine and 8am serum cortisol.

These results for adrenal suppression at steady-state are similar to those found with budesonide versus fluticasone propionate given by pressurised metered dose inhalers at steady-state, in asthmatics[231] and healthy volunteers[183]. This is perhaps not surprising given the similar pharmacological and pharmacokinetic properties of triamcinolone acetonide and budesonide. Indeed when compared on a microgram equivalent basis in a dose-response study (400-1600μg per day) there was no significant difference between the two drugs at any dose for measures of serum or overnight urinary cortisol[317]. Furthermore, in keeping with this study, there was no significant difference with placebo and triamcinolone acetonide at any dose.

In the study comparing flunisolide and triamcinolone acetonide, fractionated overnight and early morning collections of urinary cortisol corrected for creatinine excretion were measured, as these have been shown to be as sensitive as a full 24 hour uncorrected

urinary cortisol collection[185]. When looking at overnight and early morning corrected urinary cortisol/creatinine excretion, the results from this study are also in keeping with those of McIntyre et al[185], who showed that this collection is more sensitive than early morning serum cortisol. McIntyre et al[185] also showed that high dose (2000μg/ day) but not low dose (800 μg per day) inhaled beclomethasone dipropionate produced significant suppression of both overnight and early morning corrected urinary cortisol/creatinine. This study showed that only the highest recommended doses of both drugs (TAA 1600μg per day and FN 2000μg per day) produced suppression of basal adrenocortical activity, as measured by corrected urinary cortisol/creatinine, although these effects were not associated with any blunting of cortisol response to ACTH stimulation. It also demonstrated that fractionated overnight and early morning urinary cortisol excretion, corrected for creatinine, were more sensitive than either a spot 8am serum cortisol sample or the cortisol response to low dose ACTH stimulation.

It is interesting to compare the results for overnight urinary cortisol creatinine ratio from the first two studies. Both compared triamcinolone acetonide at a dose of 800μg twice daily via the same inhaler device after the same duration of therapy. However, in the first study there was no significant difference between placebo and triamcinolone, however, there was a significant difference in the second study. This is most likely due to the fact that in the first study asthmatic patients were studied and healthy volunteers were recruited into the second. This highlights the importance of evaluating asthmatic patients who would be normally taking the drugs in order to make absolute statements.

More importantly it highlights the fallacy of making direct comparisons between two studies with different samples.

The finding of a 2:1 ratio of adrenal suppression with high dose fluticasone and triamcinolone acetonide are in keeping with a chronic dosing study by Brus[318] which assessed the AUC_{24} cortisol suppression of 1000μg twice daily of a number of inhaled corticosteroids in healthy volunteers. This showed that fluticasone caused 79% suppression compared to 25% suppression with triamcinolone acetonide. However, the reported suppression of flunisolide (7%) by Brus[318] is contrary to the second study in this chapter which showed no difference between these two drugs. The study in this thesis could have been limited by power and with further patients a significant difference may have been found.

In a study comparing placebo, triamcinolone acetonide (200μg four times daily and 400μg twice daily) and fluticasone propionate (88μg and 220μg twice daily) versus prednisolone (10mg), high dose triamcinolone acetonide caused significantly more suppression than placebo or low dose fluticasone propionate using 8 hr AUC[319]. This is in contrast to the results of the first study in this chapter. The difference is hard to explain as both studies employed asthmatic patients of similar severity and both used sensitive endpoints. The duration of the study was different with 9 days in this study and 28 days in the study by Li et al[319]. The most likely difference is due to patient inhaler technique. In the study by Li et al[319], patients were "instructed in the proper use of a

metered dose inhaler", however, in all of the studies in this thesis the patients had their inhaler technique checked, using a metered dose inhaler training device, at each visit. This is likely to result in better lung delivery and systemic effects of fluticasone. Intensive training is likely to have less of an effect on triamcinolone acetonide as a result of its integrated spacer-actuator attachment.

Sorkness et al[320] compared fluticasone propionate (100μg and 500μg twice daily) and triamcinolone acetonide (300μg and 500μg twice daily) in one study and fluticasone propionate (100μg and 250μg twice daily) with flunisolide (500μg twice daily) in a second study and found no difference between active treatments and placebo in terms of the 6 hour ACTH stimulation test. This probably reflects the insensitivity of the 6 hour ACTH stimulation test

Surprisingly, there are only limited published data on the HPA-axis effects of inhaled triamcinolone acetonide and flunisolide. In a parallel group study of steroid naive mild asthmatics, sequential cumulative doubling doses of triamcinolone acetonide (800-3200 μg per day) and flunisolide (1000-4000μg per day) were administered with dose increments at weekly intervals[321]. In terms of 24 hour uncorrected urinary cortisol excretion, triamcinolone acetonide had no detectable effect at 800μg per day but produced 7% suppression at 1600μg per day, as compared to 13% and 15% suppression with 1000μg per day and 2000μg per day respectively of flunisolide. The authors concluded that relative potencies of the triamcinolone acetonide, flunisolide and

beclomethasone dipropionate appeared to be approximately equivalent for both topical and systemic effects. In another study of healthy volunteers[322], 3.5 days treatment with triamcinolone acetonide 2000μg per day and flunisolide 2000μg per day produced no significant effect on uncorrected 24 hour urinary cortisol excretion.

The low dose (0.5 μg) of ACTH was chosen in the present study as this is known to be a better reflection of the physiological stress response, as compared with the high (250 μg) dose of ACTH[188]. However, after 3 days of treatment with twice daily dosing, there was no blunting of the cortisol response to ACTH stimulation. It is possible that, with more prolonged treatment, it is possible that blunting of the cortisol response to ACTH stimulation may occur as a consequence of adrenocortical atrophy. However, it has been shown that after 3 days of inhaled budesonide 1000μg twice daily, there is evidence of an attenuated cortisol and ACTH response to stimulation with a 100μg bolus of corticotropin releasing hormone[323].

The results above look at mean figures but it is probably more clinically relevant to look at the individual systemic response to inhalation of corticosteroid. When looking at clinically relevant treatment doses less than 1000μg per day, in the first study it can be seen that there were three times as many individual results with an abnormal low values for overnight urinary cortisol excretion with fluticasone propionate than triamcinolone acetonide. However, there was no significant difference between the number of low values when comparing triamcinolone acetonide and flunisolide with either 8am or

overnight urinary cortisol. This is important to the physician when prescribing inhaled corticosteroids to individual patients as there is no simple way of distinguishing which patients will or will not have abnormal urinary cortisol levels at a given dose. In this respect it is known that there is good correlation between urinary cortisol excretion and serum cortisol response to ACTH stimulation in patients receiving inhaled corticosteroid therapy[188].

This study has also shown that, for adrenal suppression, fluticasone via pMDI plus spacer exhibited approximately 5-fold greater systemic activity than the same dose delivered via a dry powder device, and consequently a 5-fold increase in lung delivery given the linearity of the dose-response curve for fluticasone at this dose. This is explained by in vitro impactor data showing that, for fluticasone propionate, a pMDI delivers twice the respirable fraction of a dry powder device[172], and the use of a spacer doubles the lung delivery compared to a pMDI alone[324,325].

The difference in lung delivery of fluticasone propionate, when delivered by a pMDI or Accuhaler, are in keeping with the Dose of Inhaled Corticosteroids with Equisystemic Effects (DICE) study sponsored by the Asthma Clinical Research Network (Reported by M. Craft at the American Academy of Asthma Allergy and Immunology Meeting, San Diego, March 2000). The study investigated the dose of each inhaler and steroid combination required to cause a 10% fall in 12hr AUC serum cortisol and found fluticasone propionate via pMDI could be given at a quarter of the dose to cause the same suppression as fluticasone propionate dry powder. In another study[326], comparing

1mg twice daily of fluticasone propionate via a pMDI or Accuhaler, a potency ratio of 1:0.32 was determined for effects on AUC plasma fluticasone propionate.

It is important to highlight the fact that triamcinolone acetonide was compared on a microgram equivalent basis with both fluticasone propionate and flunisolide. There are several reasons for doing this. Firstly, at the time of performing the study there were no dose-response studies assessing either the systemic activity or clinical efficacy of these drugs on the steep part of the dose response curve. The only data which provided information regarding the potency of these drugs were from *in vitro* studies which have limitations as discussed above (see section 1.3.2). As it was not possible to know the relative clinical effectiveness, it is a sensible starting point to compare on a 1:1 dose ratio. Secondly, clinicians often change patients from one drug to another drug without making any change of dose. Thirdly, these dose-response studies enable comparisons to be made between different doses of different corticosteroids as well as within dose comparisons. For example, comparisons can be made between a low dose of one drug and a high dose of another. Furthermore, potency of the drug is only one variable in the propensity for adverse effects and, as has been shown in the third study, drug delivery is at least as important. In this respect, the Azmacort pMDI with integrated tube spacer delivers approximately two fold greater respirable fraction than Flixotide pMDI, as shown by data from respective *in vitro* studies using an Anderson sampler (69% v 34%)[172]. However, as the results from this study produced dose response curves which were not parallel, it was not possible to calculate potency ratios. Further studies are required to be performed comparing fluticasone propionate with higher doses of

triamcinolone in order to create parallel curves and produce a potency ratio.

CHAPTER 4

DOSE RESPONSE COMPARISON FOR RELATIVE SYSTEMIC EFFECTS OF INHALED AND ORAL CORTICOSTEROIDS

Study 1 A comparison of the systemic effects of oral prednisolone and inhaled fluticasone propionate in adult asthmatics

Study 2 A comparison of the systemic effects of oral prednisolone and nebulised budesonide in adult asthmatics

4.1 INTRODUCTION

In the previous chapter, studies compared the systemic bioactivity of different inhaled corticosteroids in terms of measures of adrenal function. Significant differences were shown between a potent inhaled corticosteroid (fluticasone propionate) and less potent inhaled corticosteroids (triamcinolone acetonide and flunisolide) in terms of twice daily and once daily dosing. There was no significant difference between two inhaled corticosteroids of similar potency (triamcinolone acetonide and flunisolide) in terms of basal and dynamic measures of hypothalamic-pituitary adrenal axis activity.

Although it is important to compare the effects of different inhaled corticosteroids and their devices of lung delivery, it is equally relevant to compare inhaled corticosteroids with oral corticosteroids. As physicians are aware of the adverse effects of long term oral corticosteroids, comparisons of oral and inhaled corticosteroids may allow a tangible awareness of the effects of inhaled corticosteroids.

Some patients with chronic severe asthma and chronic obstructive airways disease are not adequately controlled with conventional inhaled corticosteroid therapy and require maintenance treatment with oral corticosteroids such as prednisolone. However, long-term systemic adverse effects are a problem even when using the minimal effective maintenance dose of oral prednisolone. Although all inhaled corticosteroids are associated with dose-related systemic adverse effects[168], it is assumed that high-dose inhaled corticosteroids have a better therapeutic index than oral prednisolone. For these

reasons high-dose nebulised budesonide has been advocated as an alternative to patients who would otherwise be treated with maintenance daily oral prednisolone[327,328]. In order to obviate compliance problems with multiple actuations of metered-dose inhalers (pMDI), nebulisers are an alternative option for the delivery of high doses of inhaled corticosteroids to the lung. The problem of poor inhaler technique, which often occurs with pMDI's, is also avoided with nebulisers, as there is no need to co-ordinate actuation with inhalation. When this study was performed, budesonide (Pulmicort Respules, Astra Pharmaceuticals, UK) was the only suspension formulation of corticosteroid which was licensed in Europe for delivery via a nebuliser in the treatment of asthmatic patients. However, fluticasone propionate suspension (as Flixotide Nebules, Glaxo-Wellcome, Uxbridge, UK) has recently become available for nebulisation.

Prednisolone is the most widely used oral corticosteroid and is often used as a reference standard in terms of adverse and beneficial effects of anti-inflammatory medication. Fluticasone propionate is a potent inhaled corticosteroid for use in asthma. Dose-response studies have been performed comparing systemic adverse effects of inhaled fluticasone propionate with other inhaled corticosteroids (Chapter 3)[183,231] but there are no published dose-response data comparing systemic effects of oral prednisolone and inhaled fluticasone propionate.

Two studies have compared inhaled budesonide with oral prednisolone. In both of these studies, one in asthmatic[196] and the other in healthy volunteers[209], budesonide was given via a large volumatic spacer. It was therefore considered important to perform a

direct comparison of these two therapies, i.e. oral prednisolone and nebulised budesonide, which are commonly used to treat chronic severe asthmatics. From these studies the milligram equivalent potency ratio for cortisol suppression for prednisolone vs budesonide has been calculated to be 7.6:1 for steroid dependent asthmatics[196] and 5:1 for healthy volunteers[209]. Given that the glucocorticoid potency of fluticasone propionate is twice that of budesonide[151,152] a putative milligram equivalence ratio of 11:1 was chosen for comparing oral prednisolone versus inhaled fluticasone propionate and dose ratio of 5:1 for comparing nebulised budesonide and oral prednisolone. Neither of the studies is intended to investigate the therapeutic efficacy of the drugs.

4.2 METHODS

Patients

Twelve (6 female) stable mild to moderate asthmatic patients were recruited into both studies.

Study 1: mean age (SE): 28.8 (3.3) years mean forced expiratory volume in one second (FEV_1): 94.7 (3.6) % predicted, and mid-expiratory flow (FEF_{25-75}): 65.5 (6.1) % predicted. (Median dose: 300μg per day, range: 100 to 800μg per day). Eight patients were taking beclomethasone dipropionate (2 patients on 100μg per day, 3 patients on 200μg per day, 2 patients on 400μg per day and 1 patient on 500μg per day); and 4 patients were taking budesonide (1 patient on 200μg per day, 3 patients on 800μg per day).

Study 2: mean age (standard deviation): 34.7 (10.1) years mean FEV_1: 88.3 (13.2) % predicted, and FEF_{25-75} 54.8 (18.4) % predicted. Medication: beclomethasone dipropionate: n=9, fluticasone propionate: n=1, budesonide: n=2. (Median dose: 400μg per day, range: 100 to 1000μg per day).

Study Design

In, both studies a double-blind, double-dummy placebo controlled randomised crossover design was used. Spirometry was also measured at each subsequent visit, although efficacy was not an end point due to the short duration of treatment. Patients were randomised to receive either oral prednisolone (Pred) 5mg per tablet (Biorex Laboratories Ltd, Enfield, UK), or inhaled fluticasone propionate (FP) 0.11mg per

actuation (as Flovent metered dose inhaler, Glaxo-Wellcome Inc, USA) via a 750ml Volumatic spacer (Allen and Hanburys, UK) in study one; or nebulised budesonide (BUD) as 0.25 mg/ml and 0.5mg/ml (as Pulmicort Respules, Astra Pharmaceuticals Ltd., UK) given via a Ventstream nebuliser (Medicaid Ltd., UK) with mouthpiece with a Portaneb compressor (Medic-aid Ltd., Pagham, UK) delivering air at 6 l/min in Study 2.

Each drug sequence was given over a total of 12 days with six patients receiving FP or BUD first in sequence and the other six patients receiving prednisolone first in sequence. FP and BUD were given twice daily divided doses at 8am and 10pm whereas prednisolone was given orally once daily at 8am. The doses were given sequentially as follows each for four days; Pred: 1 tablet once daily, 2 tablets once daily, 4 tablets once daily (i.e. 5mg per day, 10mg per day and 20mg per day respectively); FP: 2 puffs twice daily, 4 puffs twice daily, 8 puffs twice daily (i.e. 0.44 mg per day, 0.88mg per day and 1.76mg per day respectively); BUD 2ml of 0.25mg/ml bid, 2ml of 0.5mg/ml bid, 4ml of 0.5mg/ml bid (i.e. 1 mg per day, 2 mg per day and 4 mg per day respectively). Patients received placebo tablets whilst taking FP/BUD, and inhaled placebo (MDI plus Volumatic spacer)/nebulised placebo (0.9% sterile saline) when taking Pred, using the corresponding number of tablets or number of puffs/volume of solution in order to make the trial double-dummy. Prior to each 12 day drug sequence (i.e. either FP, BUD or Pred) patients received 1 placebo tablet per day and 2 puffs bid of placebo MDI (via Volumatic spacer)/2ml vial of 0.9% saline via nebuliser, both for four days. The patients' usual inhaled corticosteroid therapy was discontinued during the placebo and treatment periods. There was also a 7 day washout between each of the 12 day treatment

sequences where patients received their usual maintenance inhaled corticosteroid therapy.

Each inhaler was discharged twice prior to use and patients used the spacer according to the manufacturers' instructions, breathing from residual volume to total lung capacity. Patients were instructed to use single puffs without delay, with each dose being followed by mouth rinsing. Prior to the study, each individual spacer was initially pre-washed in detergent, left to dry and then coated with 20 puffs. Each dose of budesonide was nebulised to residual volume (approximately 0.5-1.0ml) associated with sputtering over a period of 10 minutes. Patients were instructed to breathe at tidal volume until delivery was complete.

Measurements

After each dose level of both treatments and after both placebo periods the following measurements were made:

8am Plasma Cortisol

Serum Osteocalcin

Peripheral Blood Eosinophil Count.

Statistical Analysis

The studies were designed with sample size of 12 with 80% power (beta error =0.2) to detect a 20% difference in 8.00am cortisol (the primary end point) between treatments with the alpha error set at 0.05 (two-tailed). Osteocalcin was analysed geometrically in

order to normalise its distribution, as was eosinophil count in the second study.

The presence of dose-related suppression was determined using least squares regression analysis to evaluate the overall effects of all three dose levels for each drug. In the first study, regression analysis was applied to investigate whether for either drug, FP or Pred, there was a significant dose-response relationship, as percentage suppression for each of the three end points. For a given end point parallel slope analysis was then applied to both drugs together. In the presence of a significant fit for the common parallel slope with both drugs, a dose ratio was calculated for relative potency on a milligram equivalent basis. This was only possible for effects on cortisol.

In addition, all active treatments and both placebos were compared by an overall multifactorial analysis of variance (MANOVA) using treatment, dose, subject and period as factors, followed by Bonferroni's multiple range testing to obviate multiple pair-wise comparisons. The Bonferroni's multiple range test was set with 95% confidence intervals and hence any significant differences are reported at the $p<0.05$ level.

4.3 RESULTS

STUDY 1

There were no significant differences between the FEV_1 values (as % predicted) comparing placebo (PL) with low (L) medium (M) or high (H) doses of each drug: PL 89.4 Pred L:91.5, M:92.0, H:90.1; FP L:91.3., M:96.2. H:94. 1; or FEF_{25-75} values (as % predicted): PL: 65.3, Pred L:62.2, M:64.4. H:61.4; FP L:56.3, M:69.2, H:65.6.

There were no significant carryover effects between the first and second placebos in sequence using any of the systemic parameters measured: 8am plasma cortisol 415.2 vs 395.5 nmol/l eosinophils 0.33 vs 0.30 x 10^9/litre, or osteocalcin 1.0 vs 1.2 nmol/l. There were also no significant differences between the placebos prior to each treatment sequence (Pred vs FP): 8 am plasma cortisol 420 vs 390 nmol/l, eosinophils 0.27 v 0.36 x 10^9/litre, or osteocalcin 1.12 vs 1.0 nmol/l.

Dose-response relationships

Mean values for each of the three parameters for both FP and pred are shown in Table 4.1 With FP there was significant suppression at M and H for 8am plasma cortisol, at H dose only for osteocalcin, and at no dose for blood eosinophil count. This shows that the effects of FP are greater on cortisol compared to osteocalcin or eosinophils.

Regression analysis showed significant dose-response relationships for percentage suppression with each end-point for both drugs: Pred (8am plasma cortisol $p<0.005$

eosinophils $p<0.05$, osteocalcin $p<0.001$); FP (8am plasma cortisol $p<0.01$, eosinophils $p<0.05$, osteocalcin $p<0.05$). This showed a dose-ratio for relative potency of 8.5:1mg (95% CI 5.7 to 11.2) in terms of milligram equivalence for comparison of Pred:FP [Figure 4.1]. It was not possible to calculate a dose-ratio for either eosinophil count or osteocalcin.

Response ratios showed no significant differences at any dose level for effects on 8am plasma cortisol or eosinophils but a significant difference in osteocalcin at medium and high doses [Table 4.2]

Individual data [Figure 4.2] showed no significant difference in the numbers of individual results with abnormal low values for 8am cortisol (<150 nmol/l or <5.4μg/dl) comparing all doses of both drugs: FP (n=9/36) vs Pred (n=15/36) (p=0.2 1).

There was no significant correlation between 8am plasma cortisol and the degree of airway calibre as FEV_1 % predicted, with either FP or Pred at any dose level.

TABLE 4.1

Mean (SE) for prednisolone (Pred) and fluticasone propionate (FP) and pooled placebo for: 8 am plasma cortisol, osteocalcin and eosinophils at low, medium and high dose levels. Asterisk denotes significant difference from placebo.

	Placebo	**Low Dose**	**Medium Dose**	**High Dose**
		Pred 5 mg/day **FP 0.44 mg/day**	**Pred 10 mg/day** **FP 0.88 mg/day**	**Pred 20 mg/day** **FP 1.76 mg/day**
8am plasma cortisol (nmol/l)	405.3 (24.3)	294.1 (34.5)* 321.4 (14.7)*	230.6 (37.9)* 241.5 (31.5)*	134.9 (43.3)* 177.8 (43.6)*
Osteocalcin (nmol/l)	1.11 (0.05)	1.02 (0.05) 1.09 (0.05)	0.82 (0.04)* 1.02 (0.05)	0.68 (0.03)* 0.92 (0.05)*
Eosinophils ($x10^9$/l)	0.31 (0.04)	0.24 (0.04) 0.32 (0.05)	0.25 (0.05) 0.28 (0.05)	0.13 (0.04)* 0.21 (0.05)*

TABLE 4.2

Response ratios shown as fold difference (95% CI for difference) for prednisolone (Pred) v fluticasone propionate (FP) for: 8am plasma cortisol, osteocalcin and eosinophils at low, medium and high doses. Confidence intervals which exclude unity show a significant ($p<0.05$) difference between the two drugs at a given dose level.

	Low Dose	**Medium Dose**	**High Dose**
	Pred 5 mg/day vs FP 0.44 mg/day	**Pred 10 mg/day vs FP 0.88 mg/day**	**Pred 20 mg/day vs FP 1.76 mg/day**
8am plasma cortisol	1.2 (0.5 - 2.8)	1.1 (0.5 - 2.5)	1.5 (0.6 - 3.4)
Osteocalcin	1.1 (0.9 - 1.3)	1.2 (1.0 - 1.6)	1.4 (1.1 -1.7)
Eosinophils	1.3 (0.7 - 2.5)	1.1 (0.6 - 1.9)	1.5 (0.8 - 2.7)

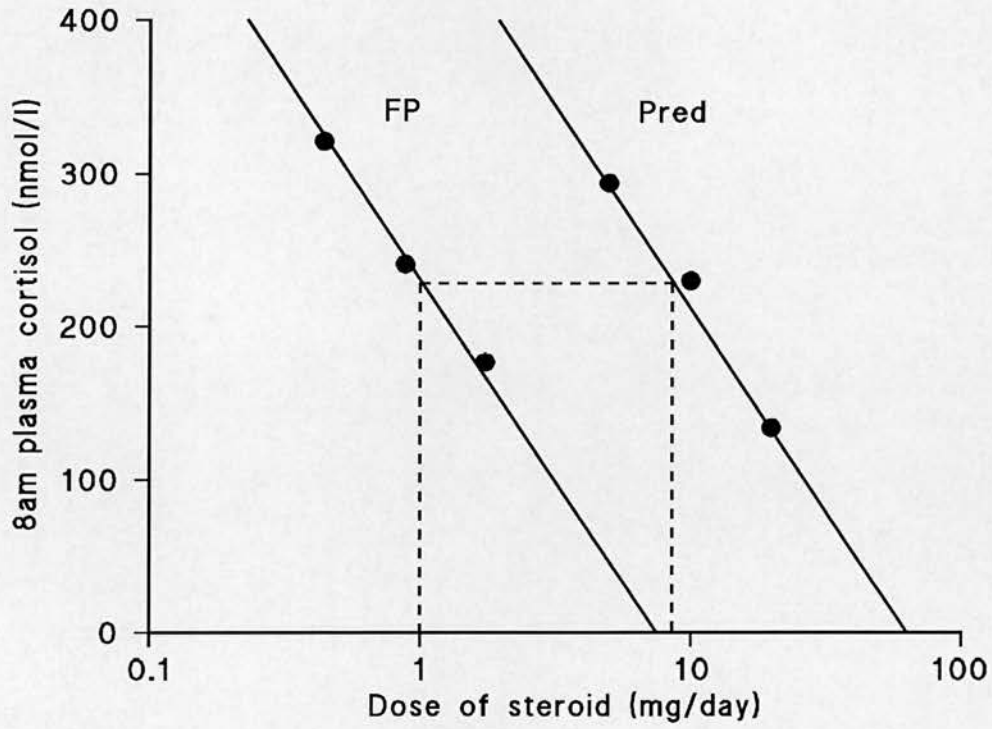


Figure 4.1
Log dose-response plot for 8am plasma cortisol suppression to show dose-ratios for relative potency. Doses of oral prednisolone (Pred) were 5mg per day, 10mg per day and 20mg per day. Doses of inhaled fluticasone propionate (FP) were 0.44mg per day, 0.88mg per day and 1.76mg per day. Parallel fitted slope analysis was used to calculate the equivalent dose of prednisolone causing the same degree of suppression as compared to 1mg of fluticasone. The relative dose ratio for Pred vs FP was calculated at 8.5:1 mg (95% CI 5.7 to 11.2).

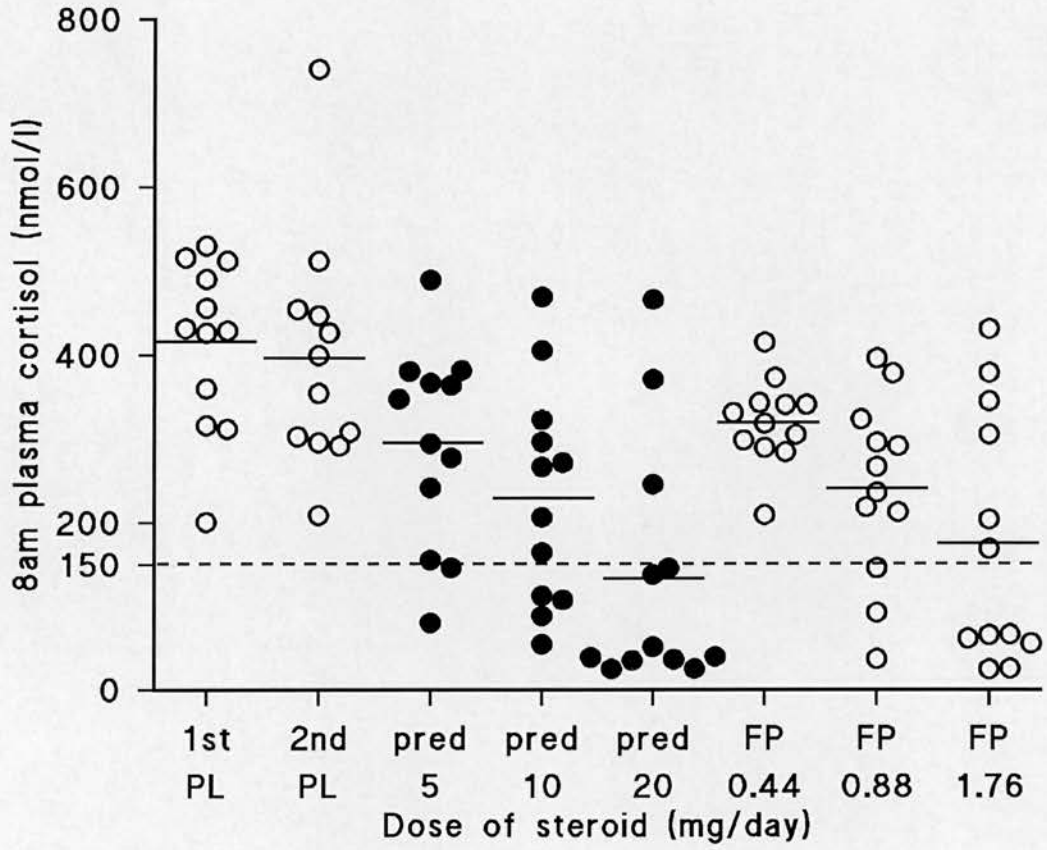


Figure 4.2
Individual values for 8 am plasma cortisol with each treatment. Horizontal bars represent mean values. The interrupted line represents the lower end of the normal reference range at <l50nmol/l (or <5.4μg/dl). There was no significant difference between fluticasone and prednisolone in terms of the number of abnormal values: n=9/36 for fluticasone vs n=15/36 for prednisolone (p=0.21).

Study 2

There were no significant carryover effects between the first and second placebos given in sequence using any of the parameters measured: 8am plasma cortisol 420.0 vs 373.4 nmol/l, eosinophils 0.35 vs 0.31 x 10^9/litre, or serum osteocalcin 0.62 vs 0.55 nmol/l. There were also no significant differences between the placebos prior to each treatment (i.e. prior to Pred or prior to BUD): 8 am plasma cortisol 406.9 vs 386.6 nmol/l, eosinophils 0.36 v 0.31 10 x 10^9/litre, or serum osteocalcin 0.61 vs 0.56 nmol/l.

There were no significant differences between the FEV_1 values (as % predicted) comparing placebo (PL) with low (L) medium (M), high (H) doses of each drug: PL 86.8%, Pred L:84.3%, M:78.1%, H:85.4%; BUD L:89.6%, M:90.43% H:92.3%; or FEF_{25-75} values (as % predicted): PL: 48.5% Pred L:47.1%, M:42.6%, H:47.7%; BUD L:52.3%, M:51.2%, H:56.3%.

8am Plasma Cortisol:

Regression analysis showed there was significant ($p<0.0001$) dose-related suppression with Pred but not with BUD ($p=0.53$) [Figure 4.3]. Compared with PL ($p<0.0005$) [Figure 4.4]

Eosinophils:

Regression analysis showed there was a significant ($p<0.001$) dose-related suppression for Pred but not with BUD [Figure 4.3]. There were significant ($p<0.05$) differences

from placebo for medium and high doses of Pred but at no dose of BUD. There were significant differences between the two drugs at the highest dose level only which amounted to a 1.87 fold difference (95% CI 1.16 to 3.00).

Osteocalcin:

Regression analysis showed there was a significant ($p<0.05$) dose-related suppression for Pred whereas this was not significant with BUD [Figure 4.3]. There were significant ($p<0.05$) differences from placebo for medium and high doses of Pred but at no dose of BUD. There were significant differences between the two drugs at the medium and highest dose levels. At the highest dose level this amounted to a 1.62 fold difference (95% CI 1.21 to 2.16).

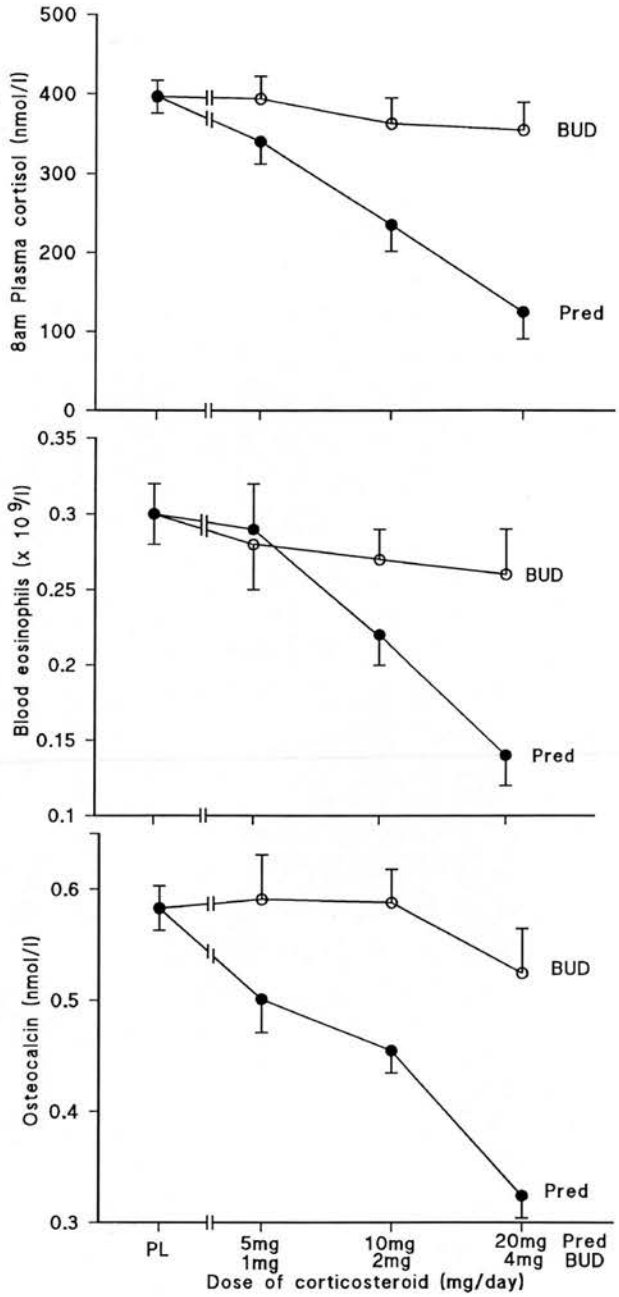


Figure 4.3

Geometric means with standard errors for pooled placebo (PL); budesonide (BUD) at 1mg per day, 2 mg per day and 4 mg per day 400; and prednisolone (Pred) at 5mg per day, 10mg per day for 8am plasma cortisol (top), blood eosinophils (middle) and serum osteocalcin (bottom). Regression analysis showed significant dose-related suppression for prednisolone (*** $p<0.0001$) for plasma cortisol, (** $p<0.001$) for blood eosinophils and (* $p<0.05$) for osteocalcin, but not a significant dose-response effect for budesonide.

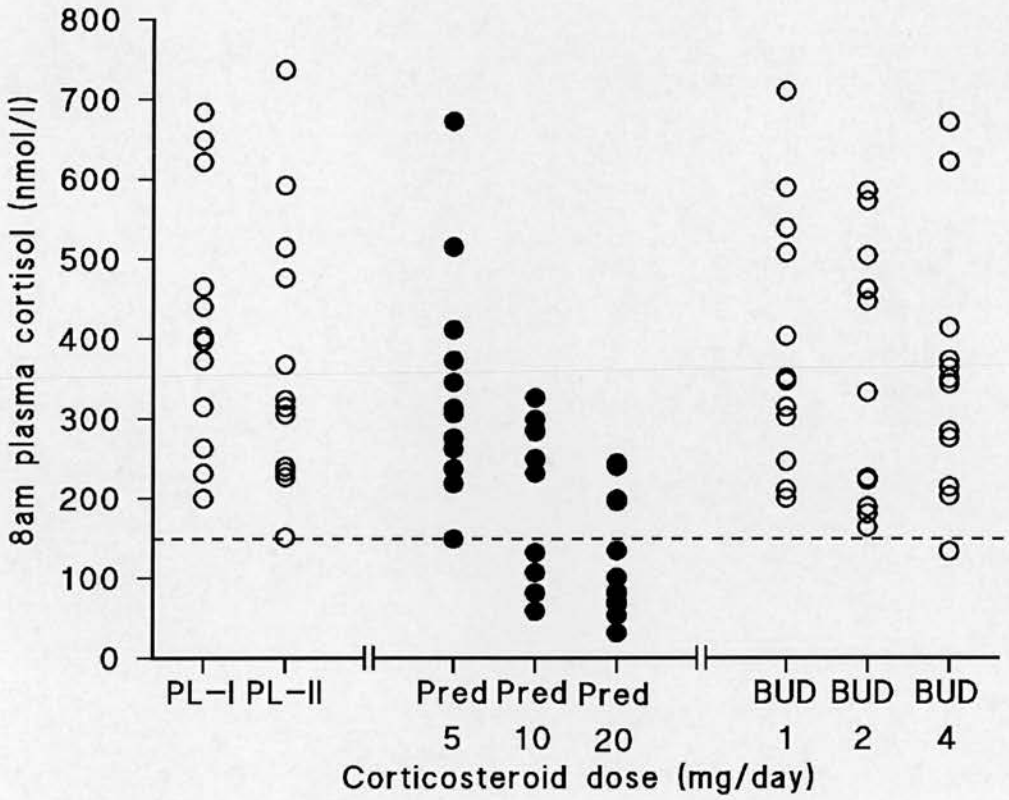


Figure 4.4
Individual values for 8 am plasma cortisol for pooled placebo (PL); budesonide (BUD) at 1mg per day, 2 mg per day and 4 mg per day 400; and prednisolone (Pred) at 5mg per day, 10mg per day. The interrupted line represents the lower limit of the normal reference range at <150nmol/l (5.4μg/dl).

4.4 DISCUSSION

These results in asthmatic patients showed that there were no significant differences in the degree of cortisol suppression exhibited by oral prednisolone and inhaled fluticasone propionate, when administered on an 11:1 mg equivalent nominal basis. Furthermore, there was no significant difference in the number of individual low values (<150nmol/l or 5.4μg/dl) between oral prednisolone and fluticasone propionate when all doses were compared. When the same data were fitted by parallel slope analysis, an 8.5:1 mg relative potency ratio for oral prednisolone compared with fluticasone propionate was found. It should be pointed out that the potency ratios for effects of fluticasone propionate and prednisolone were calculated on the raw data and not on the mean responses. Only after it was shown that the log-dose response curves were linear and that the curves were parallel was it possible to go on to calculate potencies. This was performed according to the dose-response equation and confidence intervals were generated.

Unfortunately the potency ratio for fluticasone propionate and prednisolone in terms of their effects on osteocalcin or eosinophils could not be calculated as the data did not lie on the steep part of the dose response curve for observed effects. However, it could be argued that it would not be clinically relevant to evaluate doses of fluticasone propionate greater than 1.76mg per day as this is the highest recommended dose by the manufacturers. The findings with fluticasone propionate are in keeping with Jennings et al[209] and Toogood et al[196], using budesonide who also showed greater suppression

with cortisol compared with the effects on eosinophils or osteocalcin. This is reassuring in that the bone appears to be less sensitive than the adrenal gland to the systemic effects of inhaled fluticasone propionate. In this respect Hodsman et al[208] showed that budesonide had less of an effect on bone metabolism than prednisolone for a given change in adrenal function.

The results of the studies for prednisolone are in keeping with those of Toogood et al[196] and Jennings et al[209], who also showed dose-related suppression with oral prednisolone for all the measured parameters including 8am plasma cortisol, blood eosinophils and serum osteocalcin. However, this was not the case for nebulised budesonide, which showed no significant dose-related suppression. It can be seen from the graphs of dose-response curves that for all of the endpoints, nebulised budesonide caused very little suppression even at the highest dose of 4mg per day. If higher doses of budesonide had been used, it may have been possible to detect systemic activity, but the doses chosen represented those most commonly used in clinical practice. Although it is sometimes necessary to prescribe doses greater than 4mg per day, and indeed nebulised Pulmicort is licensed as such, this is rarely done in normal practice.

The explanation for the lack of detectable systemic effects with budesonide is unlikely to be due to differences in asthmatic severity between this and previous studies[196,209]. As, in the previous studies both severe steroid dependant asthmatics[196] and healthy volunteers[209] were involved. The duration of treatment was shorter in this study but, as the effects of corticosteroids on adrenal suppression may become detectable within 12

hours of a single dose[232], this is unlikely to account for the observed differences. Furthermore steady-state blood levels would be achieved within the 4-day dosing period. The patients in this study received budesonide via a nebuliser as this is a common way of delivering high doses of inhaled medication, whereas in the previous studies a pressurised metered-dose inhaler plus large volume spacer was used. The lack of detectable systemic activity with steroids delivered by a nebuliser has previously been reported in a study where a single 4mg dose of inhaled budesonide, given via a Sidestream nebuliser (Medic-aid Ltd., Pagham, UK), had no effect on 9am serum cortisol[329].

The Ventstream nebuliser was specifically chosen because of its superior in vitro and in vivo performance characteristics compared with other conventional jet nebulisers[330,331]. Indeed the Ventstream has been shown to produce 80% of respirable particles with a diameter <5μm, as well as increasing the lung dose to the patient by boosting respiratory delivery and minimising expiratory wastage[330]. For example, in comparison to a Hudson updraft II nebuliser, the Ventstream nebuliser produces 25% more respirable particles in vitro and a two-fold improvement in lung delivery in vivo[330]. It is, therefore, not possible to justifiably explain the lack of systemic bioactivity with budesonide solely on the basis of poor nebuliser performance, although it is likely that the lung dose and hence systemic bioavailability would probably be greater from a large volume spacer. Clark and Lipworth[231] have previously shown that chronic dosing with 2mg per day of budesonide given by metered-dose inhaler to asthmatic patients,

produces no significant detectable activity on 8am plasma cortisol or overnight urinary cortisol/creatinine excretion.

The inter-individual variability in systemic activity, which can be seen by the dispersion of the values in Figure 4.2 and 4.4, may be related to effects of airway calibre inhaler technique or glucocorticoid receptor responsiveness. The high degree of first-pass metabolism of the swallowed dose of the inhaled corticosteroids will result in the systemic bioactivity being predominantly determined by the lung bioavailability, as there is no first-pass metabolism in the lung[169]. It is important, therefore, to assess whether airway calibre was altered by fluticasone and budesonide treatment, as this might conceivably result in attenuated systemic bioavailability as a result of reduced lung delivery. In this respect, there were no differences in either FEV_1 or FEF_{25-75} between the inhaled corticosteroids and prednisolone. Altered lung delivery is, therefore, unlikely to explain their relative systemic effects. The efficacy of the corticosteroids was not an end point in this study because the duration of treatment was not long enough to evaluate beneficial effects. Furthermore, the patients reported here, had well controlled mild to moderate asthma, and were therefore probably at the top of the dose-response curve for corticosteroid efficacy[168].

Great care was also taken to eliminate possible differences in inhaler technique when using the spacer or nebuliser. Indeed it was evident that even with oral prednisolone there was considerable variability in suppression, suggesting that factors other than inhaler technique and lung bioavailability are important in determining systemic

bioactivity. For example even at a high dose of 20mg per day of prednisolone there are clearly a proportion of patients who are relatively insensitive to adrenal suppression. This is more likely to represent tissue specific differences in glucocorticoid metabolism or possibly individual glucocorticoid receptor responsiveness[175].

It is also worth noting that the dosing schedules of the two drugs may have influenced the diurnal profile for adrenocortical activity, in that prednisolone was given once daily in the morning and the inhaled corticosteroids were in the morning and evening. Enteric coated prednisolone was specifically chosen, as this is the most commonly prescribed formulation in the local area. In a study evaluating the pharmacokinetic profile of enteric coated prednisolone, there was a lag in absorption such that there was an appreciable concentration remaining at 24 hours after dosing[332]. Furthermore, the corresponding 24 hour plasma cortisol profile showed that the lowest value coincided with the time point at 24 hours after dosing.

When considering the effects of fluticasone and budesonide it has been shown that suppression of a spot 8am plasma cortisol sample closely mirrors the effects on an integrated 24 hour plasma cortisol profile (see Chapte[182,182,182]r 4). This is perhaps not surprising as the maximal degree of diurnal HPA-axis suppression coincides with peak levels as measured at 8am. This is especially true for fluticasone propionate as the peak to trough variability is much less than other steroids[333], reflecting the long elimination half life of 14.4 hours[158], and the time of dosing is therefore not as important with respect to cortisol suppression. Indeed, it has been shown that significant adrenal

suppression occurs with fluticasone when administered with a 24 hour dosing interval[334]. Thus, the suppressive effects of fluticasone propionate and enteric coated prednisolone are likely to be comparable on 8am and 24 hour cortisol measurements.

The patients recruited into the studies were all taking up to 1000μg per day of inhaled corticosteroid which represented their lowest possible effective maintenance dose. These patients can therefore be considered as mild to moderate with an average FEV_1 of 88% predicted and FEF_{25-75} of 55% predicted, and would not be regularly requiring the high doses which were studied. However, it is conceivable that such patients may experience an exacerbation of their asthma and require a course of oral prednisolone and subsequently require higher doses of maintenance inhaled steroid. However, it is reassuring to know that even in asthmatics without severely impaired airway calibre, there was only minimal systemic response to high-dose nebulised budesonide.

In a study investigating the efficacy and systemic effects of prednisolone (30mg per day) and fluticasone (2mg per day and 0.5mg per day), it was shown that there was no significant difference between high dose fluticasone propionate (100% suppression of serum cortisol) and prednisolone (150%)[335]. However, in that study the clinical response was greater with high dose fluticasone propionate than prednisolone.

As the effect on adrenal function is so great with fluticasone propionate it may be considered that some of the clinical efficacy is due to a systemic prednisolone like

effect. However, this has been shown not be the case in a study by Lawrence et al[336], who demonstrated that it was the topical effect of fluticasone propionate which contributed to its activity. Fluticasone propionate was given orally at a dose which resulted in greater plasma levels than when given via the inhaled route. However, the clinical efficacy was greater with inhaled than oral fluticasone propionate. Furthermore Noonan et al[224] showed, in a sixteen week study, that in prednisolone dependant asthmatic patients taking fluticasone propionate 2mg/day, nearly 90% could be weaned off their oral corticosteroids. Indeed compared to baseline this group of patients had an average increase in FEV_1 of 0.52 litres despite a reduction in systemic activity. They found that 73% of cases had a subnormal morning plasma cortisol value (<7μg/dl) compared with 91% of cases before starting fluticasone (initial mean prednisone dose was 10.2mg/day).

O'Reilly et al[337] showed that fluticasone propionate 2mg per day via pMDI and spacer was superior to 4mg of nebulised budesonide and equivalent to 2mg nebulised budesonide in terms of peak expiratory flow rate. Furthermore, fluticasone propionate was calculated to be cheaper and more convenient to administer than nebulised budesonide.

CHAPTER 5

COMPARISON FOR RELATIVE SYSTEMIC EFFECTS OF INHALED AND INTRA-NASAL CORTICOSTEROIDS

Study 1 A comparison of systemic effects of intra-nasal triamcinolone acetonide, fluticasone propionate and beclomethasone dipropionate in healthy volunteers

Study 2 A comparison of the systemic effects of intra-nasal budesonide, mometasone furoate and triamcinolone acetonide in patients with allergic rhinitis

Study 3 A comparison of the additive systemic effects of intra-nasal plus inhaled fluticasone propionate and triamcinolone acetonide in asthmatic adult patients

5.1 INTRODUCTION

This chapter contains three separate studies which examine the systemic adverse activity of intra-nasal corticosteroids. There is no intention to offer any information regarding the clinical efficacy of the corticosteroids. The first assesses fluticasone propionate, triamcinolone acetonide and beclomethasone in healthy volunteers using 8am serum cortisol and overnight urinary cortisol. The second examines triamcinolone, mometasone furoate and budesonide in patients with allergic rhinitis using integrated 24 hour AUC serum cortisol and osteocalcin. The third study examines the additive effects of intra-nasal corticosteroids on top of inhaled corticosteroids in patients with asthma.

Intra-nasal corticosteroids have generally been regarded as being safe and free from systemic adverse effects[238,239,241,338]. They have been shown to have greater efficacy than anti-oral histamine medication[57]. Although the doses of intra-nasal corticosteroids for treatment of allergic rhinitis are small compared to inhaled corticosteroids, intra-nasal administration of corticosteroids is associated with a high level of systemic bioavailability, probably due to the abundant vascularity of the nasal mucosa and lipophilicity of modern drugs[175]. The use of modern aqueous pump sprays is associated with high intra-nasal deposition[339], although this may be partially off set by rapid nasociliary clearance into the throat. Furthermore, there is no first-pass inactivation in the nose, and so absorption of the unchanged drug occurs directly into the systemic circulation. It is now increasingly recognised with inhaled corticosteroids that detectable systemic activity occurs at doses less than 1000μg per day (see Chapter 3) particularly

with fluticasone propionate due to its specific pharmacological and pharmacokinetic properties[169]. The question therefore ensues as to whether the same effects occur with nasal corticosteroids when given in clinically recommended doses.

The first study in this chapter is designed, therefore, to compare the systemic activity of intra-nasal triamcinolone acetonide, beclomethasone dipropionate and fluticasone propionate in terms of effects on HPA-axis activity. Both basal and dynamic measures of HPA-axis activity were chosen, namely overnight urinary cortisol excretion and cortisol response to stimulation with a physiological dose of ACTH (0.5μg).

There are several reasons why the results of this study may not reflect what happens in clinical practice. Firstly, it may be more clinically relevant to assess the effects of intra-nasal medication in patients with rhinitis who would normally be taking such treatment. There may be a difference in the absorption through the nasal mucosa in patients and healthy volunteers. The absorption is likely to be decreased due to nasal obstruction and nasal secretions, and potentially increased by the hyperaemia associated with nasal inflammation.

Another concern is the measure of adrenal activity used in the first study. Adrenal suppression is commonly used as a marker for the systemic bioactivity of inhaled and intra-nasal corticosteroids[175] and can be assessed by both fractionated or 24 hour measurements of urine and plasma cortisol. As cortisol secretion varies according to the

diurnal circadian rhythm, spot samples are usually taken between 8.00am and 9.00am in order to coincide with the physiological peak blood levels. However these do not take account of the changes during the day and night, and have been shown to be less sensitive than a full 24 hour urine collection[175,184]. Thus, the second study used a 24-hour AUC marker rather than a spot serum 8am sample.

Furthermore, the first study only assessed the effects of intra-nasal corticosteroids on HPA-axis activity. As has been shown in Chapter 4, the bioactivity of corticosteroids is not the same in all tissues. Therefore a second study was performed in patients with rhinitis to assess the comparative systemic bioactivity of budesonide, mometasone furoate and triamcinolone acetonide using sensitive markers of adrenocortical activity, bone formation and blood count. All drugs were given via aqueous formulations once daily at the usual clinically recommended dose.

Given the frequency of patients with rhinitis and asthma it would seem necessary to investigate the side effect profile when these drugs are prescribed concomitantly. The aim of the third study was therefore to evaluate the integrated 24 hour and fractionated profiles for serum and urinary cortisol in asthmatic patients receiving inhaled corticosteroids given alone or in conjunction with aqueous formulations of intranasal corticosteroids. Triamcinolone acetonide (TAA) and fluticasone propionate (FP) were chosen for investigation as examples of corticosteroids with different pharmacological and pharmacokinetic properties, and both were given within the manufacturer's licensed dose range for inhaled and intranasal formulations.

5.2 METHODS

Patients

Study 1: Sixteen healthy non-allergic volunteers (9 female) of mean age (SE) 30.7 (2.7) years.

Study 2: Twenty patients (12 female) with rhinitis of mean age (SE) 35.7 (3.5) years completed the study. All but 3 patients were taking intra-nasal corticosteroids (12 taking beclomethasone dipropionate 200μg bid, 3 taking fluticasone propionate 200μg od, one taking budesonide 200μg od, and one taking flunisolide 100μg bid). Nine patients were taking oral anti-histamines and two were taking inhaled salbutamol on an as required basis. A skin prick test was performed in all patients which revealed a Grade 2 or greater (>6mm) wheal and flare reaction to grass or tree pollen in 16 patients and to house dust mite in 8 patients. One patient with perennial rhinitis also had a positive skin prick test to house dust mite.

Study 3: Twelve patients (6 female) mean age (SE): 25.9 (3.5) years mean FEV_1: 84.0 (4.0) % predicted, mean mid forced expiratory flow rate (FEF_{25-75}) 56.1 (6.0) % predicted, and a median dose of inhaled corticosteroids of 500μg per day (range 200μg per day-1200μg per day). One patient was also receiving intranasal corticosteroid at a dose of 200μg per day.

Study Design

In all studies a single (investigator) blind randomised placebo controlled design was used. The first two studies were four-way crossover and used the Williams Design

whereas the last was a 2-way crossover study.

Study 1: Subjects received four different randomised intra-nasal treatments with either: triamcinolone acetonide (TAA) 55μg per actuation (as Nasacort AQ, Rhone Poulenc Rorer Pharmaceuticals, Inc, Collegeville USA); fluticasone propionate (FP) 50μg per actuation (as Flonase, Glaxo Wellcome Inc, USA) or beclomethasone dipropionate (BDP) 84μg per actuation (as Vancenase AQ double strength, Schering Corporation, Kenilworth, USA), or placebo. Each treatment was given once daily, 2 squirts up each nostril, at 8am, for four days.

Study 2: Subjects received four different randomised intra-nasal treatments with either: TAA 220μg od (as Nasacort AQ 55μg per actuation, Rhone Poulenc Rorer Ltd., East Sussex, UK); mometasone furoate (MF) 200μg od (as Nasonex 50μg per actuation, Schering-Plough Ltd., Hertfordshire, UK) or budesonide (BUD) 200μg od (as Rhinocort Aqua 100μg per actuation, Astra Pharmaceuticals Ltd., Herts, UK) or placebo. Each treatment was given once daily at 8am for four days. BUD was administered as one squirt up each nostril, whereas MF, TAA and PL were administered as 2 squirts up each nostril.

In both of these studies was also an initial non-randomised 4 day placebo run-in prior to the randomised treatment block. The initial non-randomised placebo was compared to the randomised placebo in order to assess for any carryover effect in the randomised

treatment block. Each of the four randomised treatments were separated by a 7 day washout period.

Study 3: The drug treatment phase consisted of 5 days of active inhaler plus placebo nasal spray, followed by 5 days of active inhaler plus active nasal spray. Patients were randomised to receive either triamcinolone acetonide first or fluticasone propionate first. Triamcinolone acetonide was given as Azmacort integrated spacer actuator 100μg per actuation and Nasacort AQ 55μg per actuation, Rhone-Poulenc Rorer Inc, USA. Fluticasone propionate was given as Flovent metered dose inhaler 110μg per actuation without spacer and Flonase 50μg per actuation, Glaxo-Wellcome Inc, USA. As each inhaler was given at a dose of 8 puffs bid and each nasal spray was 2 puffs up each nostril once daily, the doses of each drug were as follows: Azmacort 1600μg per day, Nasacort AQ 220μg per day, Flovent 1760μg per day and Flonase 200μg per day. Both inhaled and intranasal drugs were prescribed according to manufacturers' recommendations including priming of the nasal sprays. Azmacort oral inhaler has an integrated actuator-spacer device, whilst the Flovent metered dose inhaler is not licensed for use with a spacer. The above inhaled corticosteroid doses refer to the dose delivered ex-actuator, in accordance with US product labeling, and are therefore less than the nominal dose (ex-value). For example Flovent 110μg per puff ex-actuator dose is equivalent to Flixotide 125μg per puff ex-valve dose, whilst Azmacort 100μg per puff ex-actuator/spacer is equivalent to 200μg per puff ex-value.

Patients were randomised in blocks to receive one of two treatment sequences: 1) nPL+inhPL, nPL+inhTAA, nTAA+inhTAA, washout, nPL+inhPL, nPL+inhFP and nFP+inhFP or 2) nPL+inhPL, nPL+inhFP, nFP+inhFP, washout, nPL+inhPL, nPL+inhTAA, nTAA+inhTAA. Where nPL=nasal placebo, inhPL=inhaled placebo, inhTAA=inhaled triamcinolone actonide 800μg twice daily, nTAA=intra-nasal triamcinolone acetonide 200μg once daily, inhFP=inhaled fluticasone propionate 880μg twice daily and nFP=intra-nasal fluticasone propionate 220μg once daily. Each patient took both an active or placebo inhaler (8 puffs bid) and an active or placebo nasal spray (2 squirts via each nostril once daily) on all 15 days of each arm of the trial.

There was a 10 day washout period between each of the 15 day treatment sequences where patients received their usual maintenance inhaled corticosteroid therapy. Prior to each 10 day drug period with the active drug (i.e. either fluticasone propionate or triamcinolone acetonide), patients received the respective matching placebo inhaler and placebo nasal spray for 5 days (making a total of 15 days for each treatment sequence). The patients' usual inhaled and intranasal corticosteroid therapy was discontinued during the placebo and treatment periods.

Measurements

Measurements were made after each 4 day period with active treatment or placebo in the first two studies; and after both placebos, inhaled alone or inhaled plus nasal treatment for both corticosteroids in the third study.

In Study 1 8am Serum Cortisol, Low dose Synacthen test, Overnight Urinary Cortisol and Overnight Urinary Cortisol/Creatinine Ratio were measured. In the other two studies 24 hour area under curve and fractionated (8am, overnight, daytime) serum plasma cortisol and 24 hour and fractionated (8am, overnight, daytime) urine cortisol/creatinine ratio were measured. In the second study only 8am serum osteocalcin and 8am blood eosinophil count were measured.

Statistical Analysis

The first study was designed with sample size of 16 with 90% power (beta error = 0.1) to detect a 20% difference in overnight urinary cortisol between treatments with the alpha error set at 0.05 (two-tailed). The other two studies were designed with sample sizes of 20 and 16 with 80% power (beta error = 0.2) to detect a 20% difference in 24 hour integrated AUC plasma cortisol and 8am plasma cortisol respectively between treatments with the alpha error set at 0.05 (two-tailed).

In order to normalise its distribution, all data were analysed by logarithmic transformation in the first and third studies; and urinary cortisol/creatinine data only in the third study.

In the first two studies, comparisons were made of all three treatments and both placebos (randomised and non-randomised) by an overall analysis of variance with subject, treatment and period as factors. In the third study Comparisons between all 4 active treatments and both placebos were made by a multifactorial overall analysis of variance,

with subject, drug, treatment and period as factors. Bonferroni's multiple-range testing was then applied in order to obviate multiple pair-wise comparisons, so as to assess where there were significant differences between treatments and the randomised placebo. The Bonferroni's range test was set with 95% confidence intervals and hence any significant differences are only reported at the $p<0.05$ level. 95% confidence intervals for the mean treatment differences were also calculated. In the third study, a comparison was also made between the first and second placebos given in sequence within the study design to check for any carry-over effect between the two drug sequences. In addition, a comparison was made of the two placebos given before FP and TAA irrespective of the treatment sequence.

In the third study, individual values for low 24 hour urinary cortisol excretion <40nmol (<14.4μg) and 8am serum cortisol <150nmol/l (<5.4μg/dl) were analysed using the Chi-Square test. A value of <40nmol for 24 hour urinary cortisol excretion and <150nmol/l for 8 am serum cortisol are considered to be abnormal values below the reference range[176].

5.3 RESULTS

Study 1

There was no significant carryover effect between the non-randomised placebo and randomised placebo respectively using any of the parameters measured (as geometric means ± SE): Overnight urinary cortisol: 17.1 ± 2.3 vs 20.8 ± 2.8 nmol, pre ACTH serum cortisol: 547.5 ± 23.2 vs 574.0 ± 24.3 nmol/l, or post-ACTH 781.2 ± 32.6 vs 761.0 ± 31.7 nmol/l. The randomised placebo was used for all comparisons with the three active treatments.

Overnight urinary cortisol excretion.

Compared with PL (20.8 ± 2.8 nmol) there was statistically significant ($p<0.05$) suppression with FP: (11.8 ± 1.6 nmol) but not with TAA (16.0 ± 2.1 nmol) nor with BDP (16.5 ± 2.2 nmol) [Figure 5.1]. This amounted to a ratio of 1:1.75 for PL versus FP (95% CI 1.01 to 3.03). There was a ratio of 1:1.30 for PL versus TAA (95% CI 0.75 to 2.25) and a ratio of 1:1.26 for PL versus BDP (95% CI 0.73 to 2.18). There were no significant differences between the three active treatments. There was also a trend towards suppression of overnight urinary cortisol/creatinine ratio (nmol/mmol) but this was not statistically significant: PL (5.2 ± 0.5), TAA (5.0 ± 0.5), BDP (4.3 ± 0.4) and FP (4.3 ± 0.4). Individual values for overnight urinary cortisol are depicted in Figure 5.1. This shows that there was considerable inter-individual variability in the propensity for suppression.

Pre-ACTH 8am Serum Cortisol: [Figure 5.2]

There were no significant differences between placebo (574.0 ± 24.3 nmol/l) and any of the other treatments: TAA: (572.3 ± 24.2 nmol/l), BDP: (590.6 ± 25.0 nmol/l), FP: (581.9 ± 24.6 nmol/l). There were no patients who had an individual value less than 150 nmol/l for any drug.

Post ACTH Serum Cortisol [Figure 5.2]

There were no significant differences between placebo (761.0 ± 31.7 nmol/l) and any of the other treatments TAA: (767.8 ± 32.0 nmol/l), BDP: (749.6 ± 31.2 nmol/l) FP: (769.7 ± 32.1 nmol/l). There were no patients who had an individual value less than 500nmol/l for any drug.

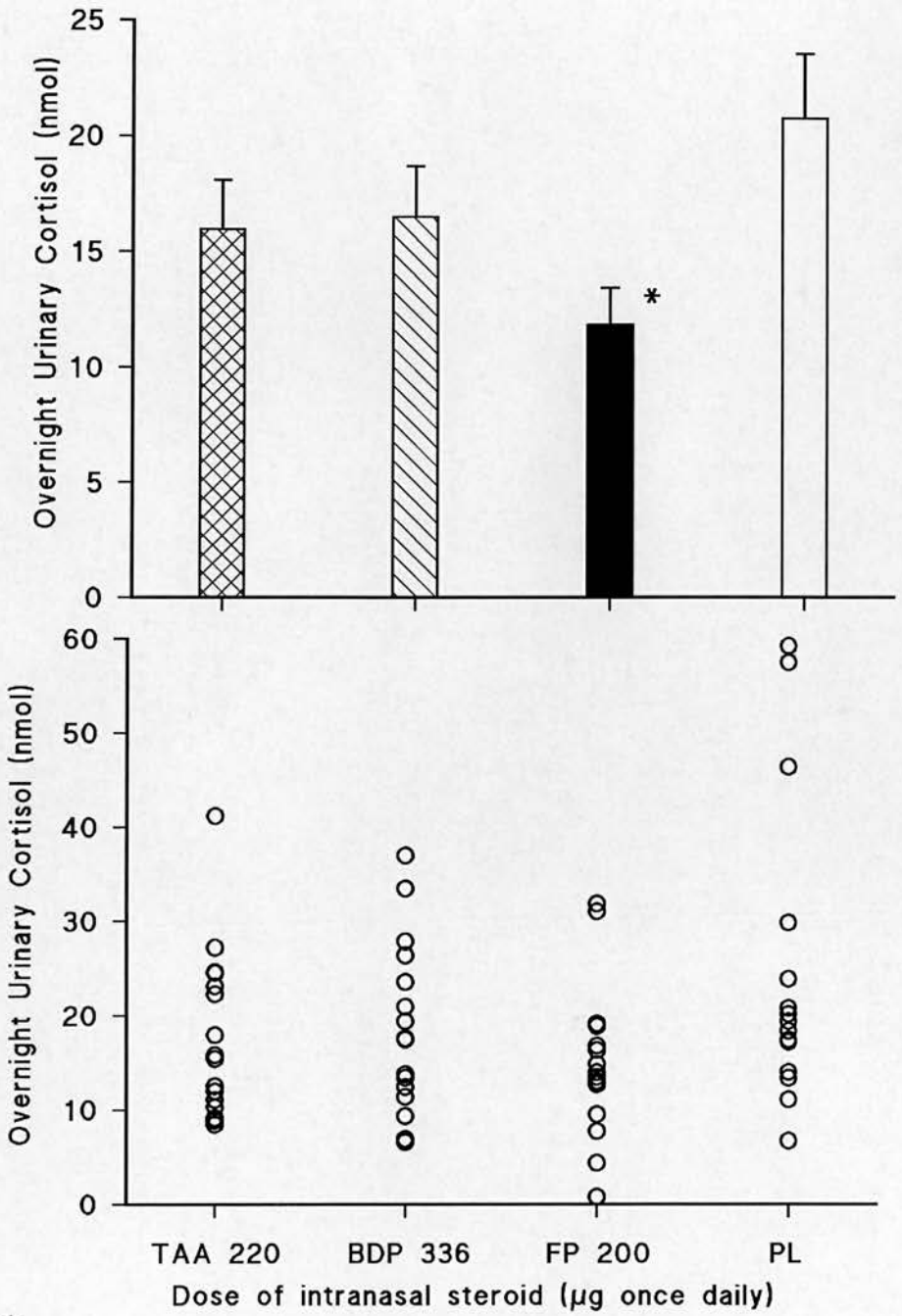


Figure 5.1:
a) Geometric mean (and SE) and b) individual values for overnight urinary cortisol excretion with intra-nasal administration of placebo (PL), triamcinolone acetonide 220µg once daily (TAA-220), beclomethasone dipropionate 336µg once daily (BDP 336) and fluticasone propionate 200µg once daily (FP 200). Asterisk denotes significant ($p<0.05$) difference from placebo.

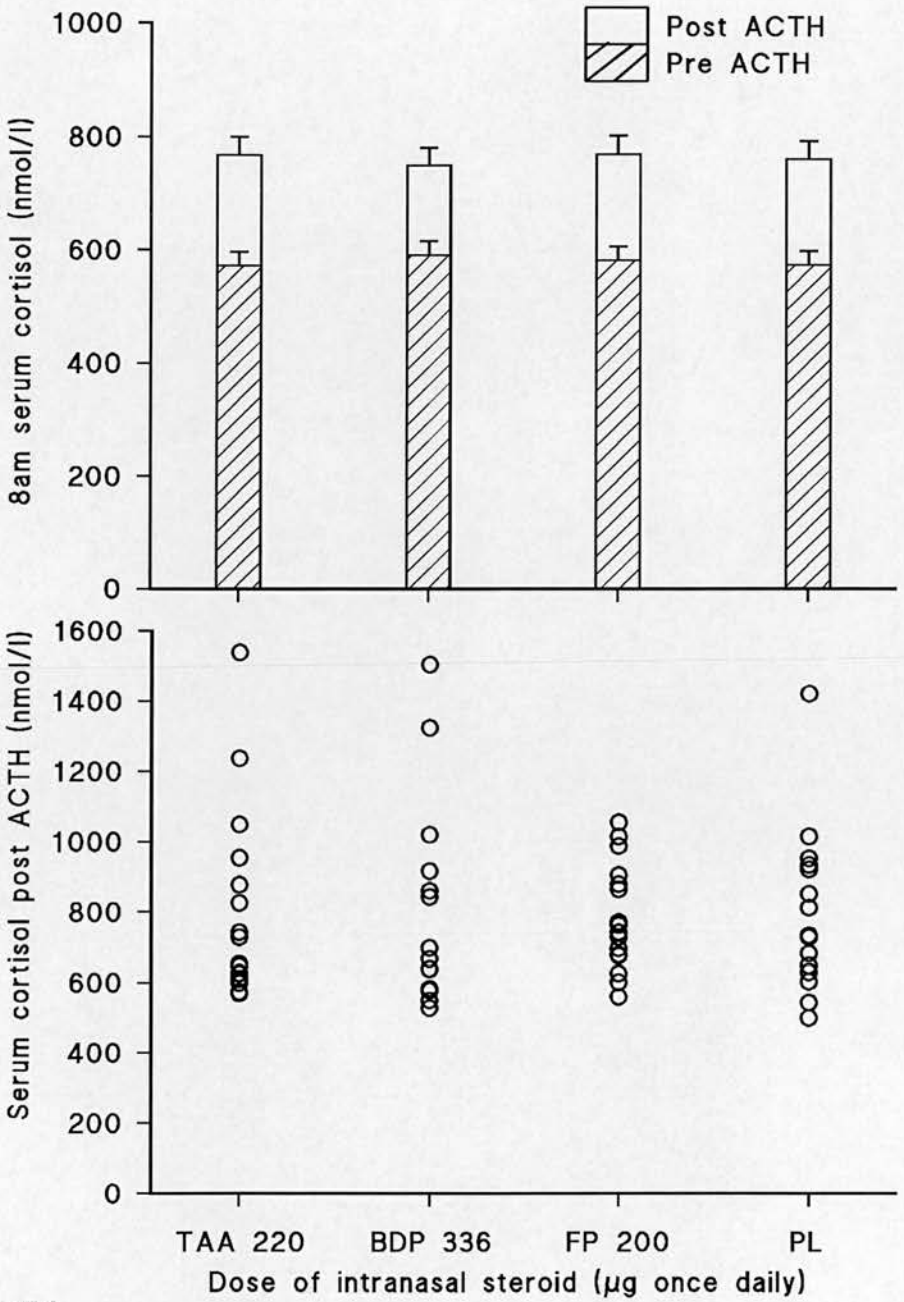


Figure 5.2

(a) Geometric means and SE for pre and post ACTH stimulated serum cortisol and (b) individual values for post ACTH stimulated serum cortisol for placebo (PL), triamcinolone acetonide 220µg once daily (TAA-220), beclomethasone dipropionate 336µg once daily (BDP 336) and fluticasone propionate 200µg once daily (FP 200). There were no patients with post stimulated cortisol less than 500nmol/l for any drug.

Study 2

There was no significant carryover effect between the non-randomised placebo and randomised placebo respectively using any of the parameters measured [Table 5.1].

Plasma cortisol.

There was no significant difference between the randomised placebo and any of the active treatments in terms of fractionated or 24 hour measurements [Figure 5.3]. From the 24 hour plasma cortisol profile, it can be seen that the normal circadian diurnal rhythm was unaltered with all drugs and the curves were virtually super-imposable with that of placebo[Figure 5.4]. Inspection of individual data shows a considerable degree of inter-individual dispersion and there were no individual values for 8am cortisol <150nmol/l (<5.44μg/l) with any of the treatments[Figure 5.5].

Urinary Cortisol

There was no significant difference between the randomised placebo and any of the active treatments in terms of fractionated or 24 hour uncorrected urinary free cortisol [Table 5.2] or for fractionated and 24 hour cortisol/creatinine [Figure 5.6]. There were two values with TAA for 24 hour urinary cortisol of <40nmol (14.4μg) and one value with MF for overnight urinary cortisol of <10 nmol (3.6μg)[Figure 5.5].

Osteocalcin

There was no significant difference between randomised PL (1.3 ± 0.1) and any of the

three active treatments (BUD: 1.2 ±0.2, MF: 1.3 ±0.2, TAA: 1.2 ±0.2).

Blood Eosinophil Count

There was no significant difference between randomised PL (0.29 ± 0.05) and any of the three active treatments (BUD: 0.27 ±0.04, MF: 0.25 ±0.04, TAA:0.24 ±0.04).

Table 5.1

Means (SE) for first placebo (PL-1) in sequence and randomised placebo (PL-R) for 24 hour and fractionated plasma cortisol and urinary cortisol/creatinine ratio, for blood eosinophil count and for serum osteocalcin. There were no significant differences between the two placebo values for any tissue marker.

	PL-1	PL-R		PL-1	PL-R
Overnight plasma cortisol (nmol.hr/l)	3071.0 (248.1)	2719.2 (238.6)	overnight urinary cortisol/creatinine ratio (nmol/mmol)	9.2 (1.0)	6.9 (0.8)
8am plasma cortisol (nmol/l)	545.1 (37.8)	585.3 (42.2)	8am urinary cortisol/creatinine ratio (nmol/mmol)	18.0 (2.2)	19.1 (2.3)
Daytime plasma cortisol (nmol.hr/l)	3414.4 (250.5)	3593.6 (365.7)	Daytime urinary cortisol/creatinine ratio (nmol/mmol)	9.8 (0.6)	10.4 (0.6)
24 hour plasma cortisol (nmol.hr/l)	6485.4 (467.9)	6312.9 (564.4)	24 hour urinary cortisol/creatinine ratio (nmol/mmol)	119.8 (7.8)	114.7 (6.7)
Osteocalcin (nmol/l)	1.5 (0.2)	1.3 (0.2)	Blood eosinophil count (x 10^9/l)	0.29 (0.04)	0.29 (0.05)

Table 5.2

Means (SE) for budesonide (BUD), mometasone furoate (MF), triamcinolone acetonide (TAA) and randomised placebo (PL) for 24 hour and fractionated (Overnight, 8am and daytime) urinary cortisol (nmol). There was no significant difference between any of the active treatments and placebo.

	PL	BUD	MF	TAA
24 Hour urinary cortisol	114.7 (6.7)	96.8 (6.1)	107.7 (6.3)	102.2 (6.0)
Overnight urinary cortisol	40.4 (4.4)	39.3 (4.4)	36.0 (3.9)	46.0 (5.0)
8am urinary cortisol	8.1 (1.4)	10.0 (1.8)	11.3 (2.0)	8.9 (1.6)
Daytime urinary cortisol	67.4 (4.3)	54.7 (3.6)	67.2 (4.3)`	56.4 (3.6)

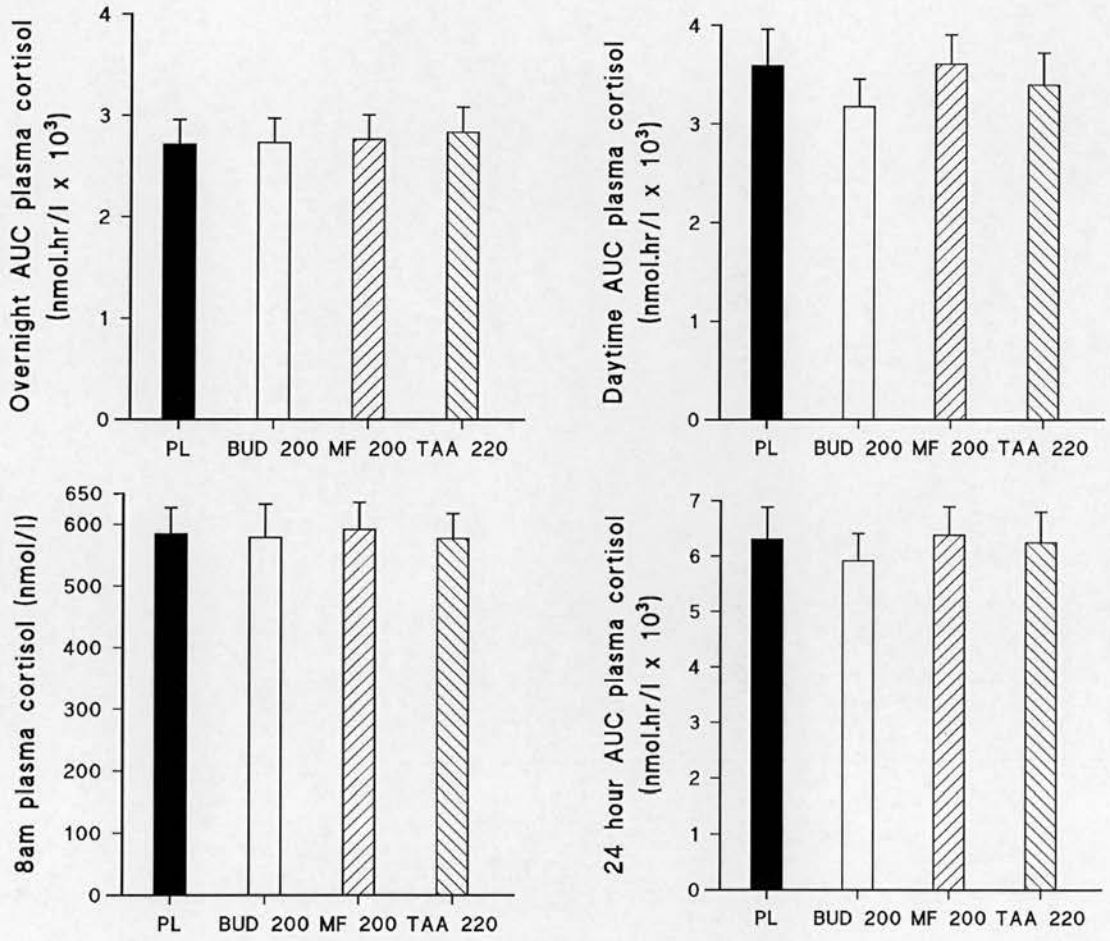


Figure 5.3
Arithmetic means with standard error for placebo (PL), budesonide 200μg once daily (BUD 200), mometasone furoate 200μg once daily (MF 200), and triamcinolone acetonide 220μg once daily (TAA 220), for effects on 24 hour AUC and fractionated (overnight, 8am and daytime) plasma cortisol. There was no significant difference between placebo and any of the active treatments.

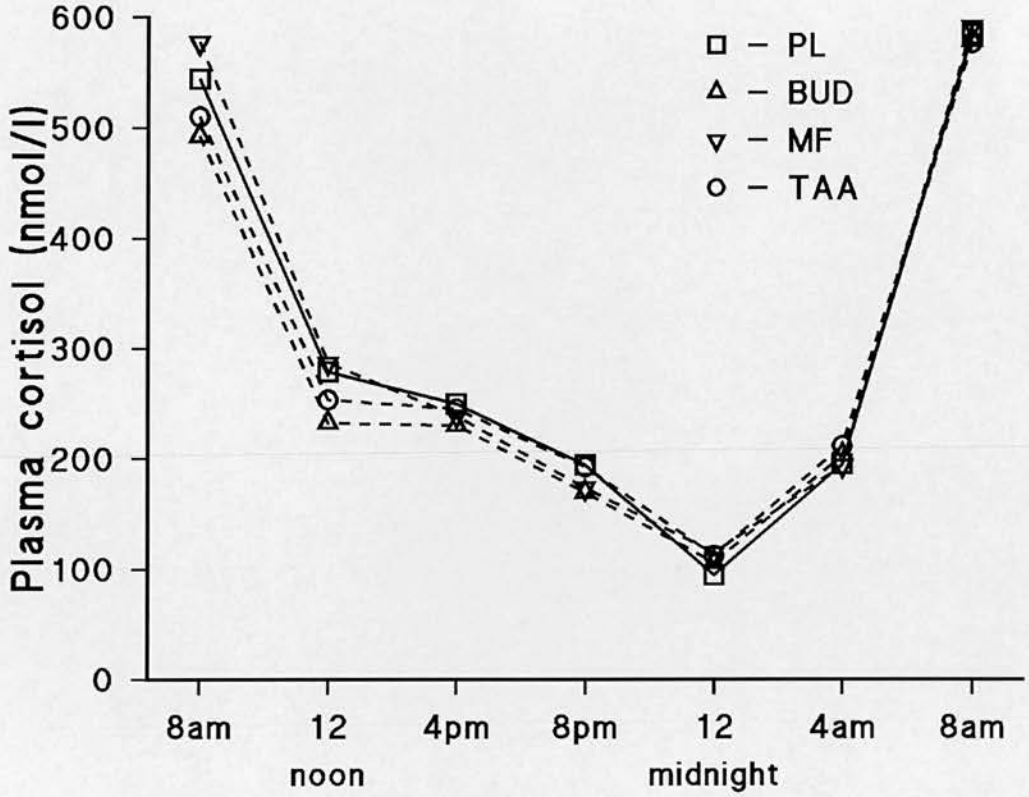


Figure 5.4
Means for 24 hour profile of plasma cortisol for placebo (PL), budesonide 200μg once daily (BUD), mometasone furoate 200μg once daily (MF), and triamcinolone acetonide 220μg once daily (TAA), for effects on 24 hour AUC and fractionated (overnight, 8am and daytime) plasma cortisol

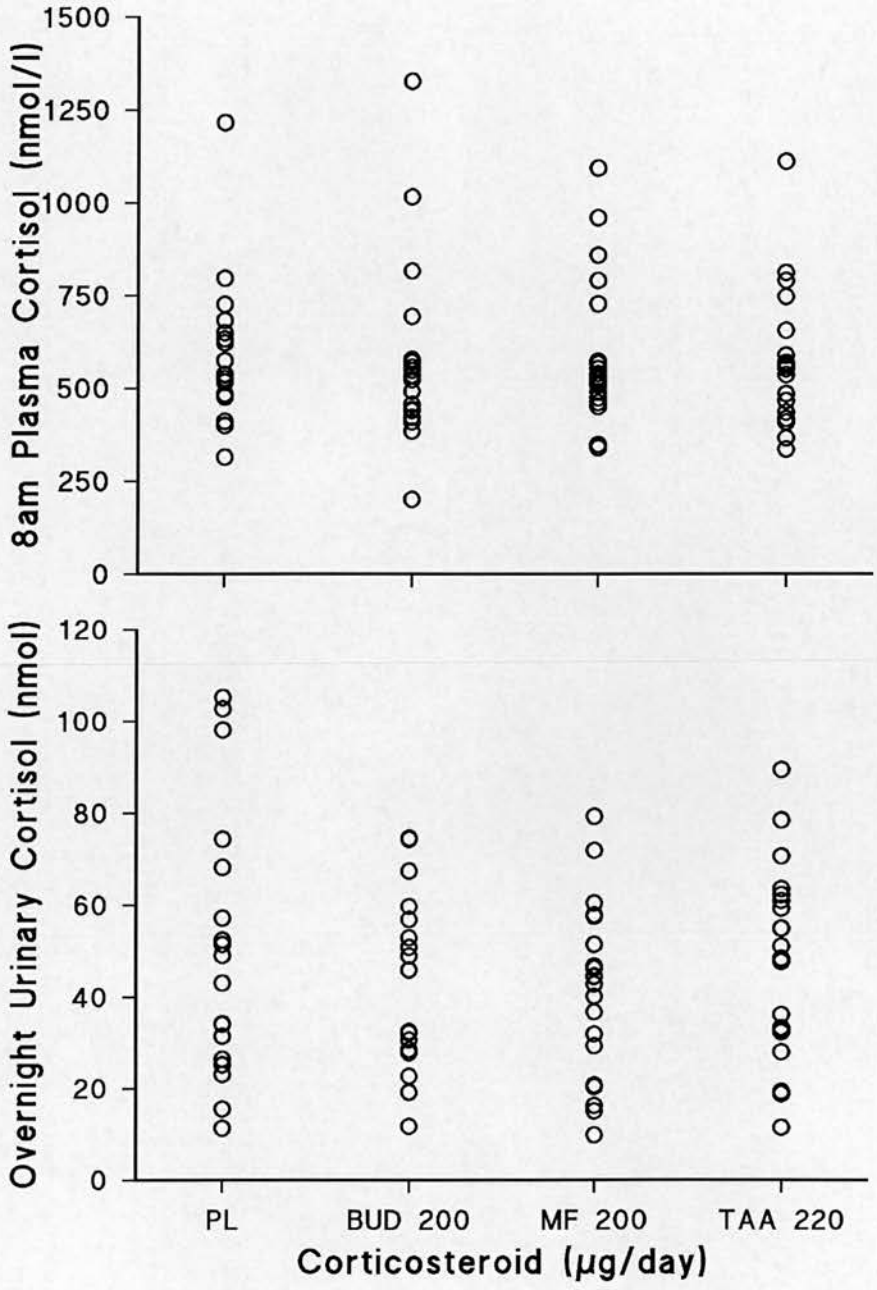


Figure 5.5:
Individual values for a) 8am plasma cortisol and b) overnight urinary cortisol for placebo (PL), budesonide 200µg once daily (BUD 200), mometasone furoate 200µg once daily (MF 200), and triamcinolone acetonide 220µg once daily (TAA 220), for effects on 24 hour AUC and fractionated (overnight, 8am and daytime) plasma cortisol. There were no valued below 150nmol/l for 8am plasma cortisol and only one value (for MF) below 10nmol for overnight urinary cortisol.

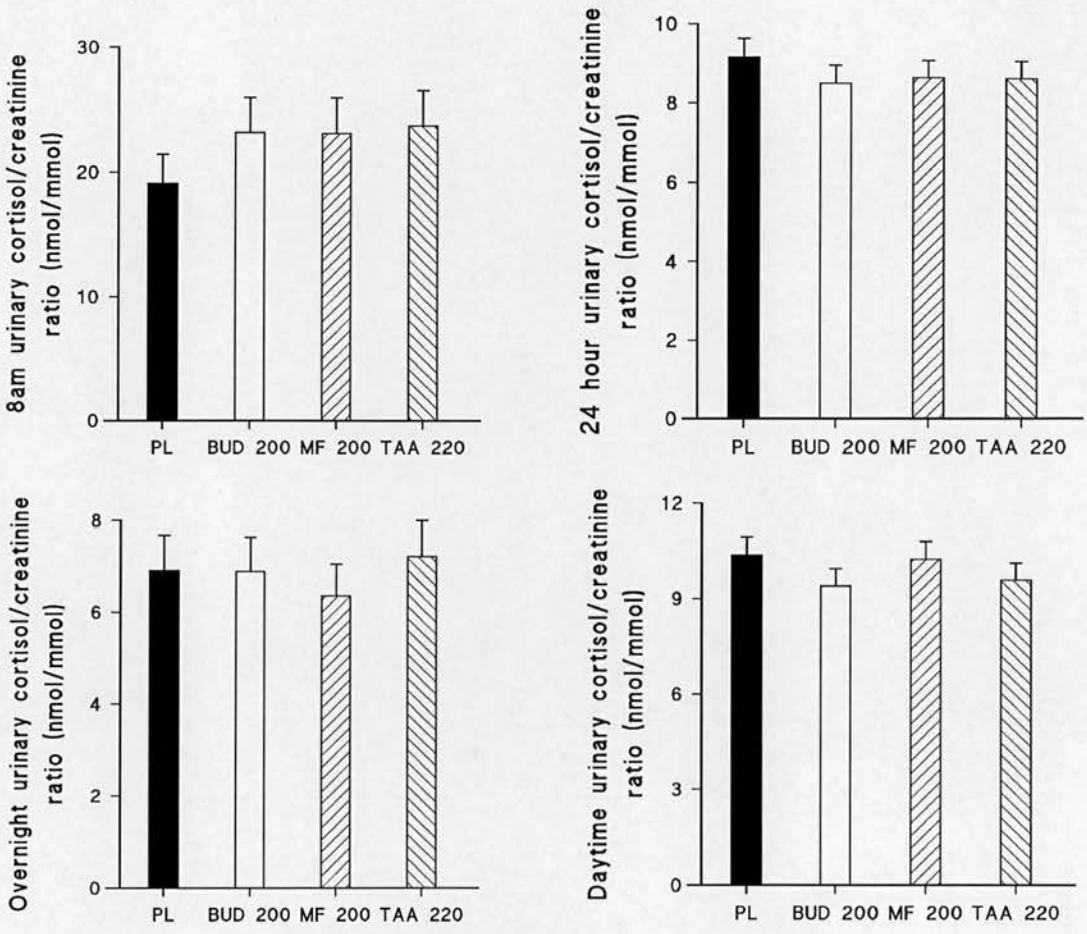


Figure 5.6:
Geometric means with standard error for 24 hour and fractionated (overnight, 8am daytime) corrected urinary cortisol/creatinine ratio for placebo (PL), budesonide 200μg once daily (BUD 200), mometasone furoate 200μg once daily (MF 200), and triamcinolone acetonide 220μg once daily (TAA 220), for effects on 24 hour AUC and fractionated (overnight, 8am and daytime) plasma cortisol. There were no significant differences between placebo and any of the three active treatments.

Study 3

There were no significant carryover effects between the first and second placebos given in sequence using any of the parameters measured [Figures 5.7, 5.8, 5.11]. Placebo values prior to treatment (irrespective of sequence) with either FP and TAA were also not significantly different (FP vs TAA): a) 24 hour serum cortisol 6880 vs 7280 nmol.hr/l, b) fractionated serum cortisol (overnight: 2590 vs 2760 nmol.h/l, 8am 587 vs 608 nmol/l, daytime: 4240 vs 4460 nmol.h/l), c) 24 hour corrected urinary cortisol/creatinine excretion 7.0 vs 6.4 nmol/mmol, or d) fractionated urine collections corrected for creatinine (overnight: 3.9 vs 3.8 nmol/mmol, 8am: 16.1 vs 16.5 nmol/mmol and daytime: 9.3 vs 9.1 nmol/mmol). Mean FEV_1 values showed a significant difference between both placebo and inhaled alone for both treatments but no difference between the two drugs for either inhaled alone or combined inhaled and intranasal: PL: 2.98L, inhTAA+nPL: 3.18L, inhFP+nPL:3.29L, inhTAA+nTAA: 3.05L, inhFP+nFP:2.94L.

Serum cortisol:

Inspection of the 24 hour serum cortisol time profile shows that the normal diurnal circadian rhythm was abolished by FP [Figure 5.7]. With TAA there was blunting of the 8 am early morning cortisol peak although the normal diurnal circadian rhythm remained preserved. For both 24 hour and fractionated serum cortisol there was a significant ($p<0.05$) difference between placebo and all of the other 4 active treatments. There was a significant difference ($p<0.05$) between FP and TAA for both inhaled medication alone and inhaled plus nasal medication [Figure 5.8 and 5.9]. For 8 am

serum cortisol for all active treatments, there was a significant ($p<0.0005$) difference in the number of individual values between FP [17/24 (71%)] and TAA [3/24 (12%)] with an abnormal low level <150nmol/L [Fig 5.10]. The addition of intranasal corticosteroid did not produce any further significant suppression of mean serum cortisol values.

Urinary cortisol/creatinine:

For 24 hour and fractionated measurements there was a significant ($p<0.05$) difference between placebo and all of the other active treatments and a significant difference ($p<0.05$) between FP and TAA for both inhaled medication alone and inhaled plus nasal medication. [Figure 5.9 and Figure 5.11]. The 95% CI for a within-subject differences showed less variance for urine than for serum measurements [Figure 5.9]. For 24 hour urinary cortisol excretion [Figure 5.10], there was a significant ($p<0.0005$) difference between those treated with FP [17/24 (71%)] and TAA [4/24 (16%)], when analysing the number of individual values with an abnormally low level <40nmol. The addition of nasal corticosteroid did not produce any further significant suppression of mean or urinary cortisol/creatinine values. However, the addition of intranasal FP resulted in 3 more abnormal values for 24 hour urinary cortisol, as compared to inhaled FP alone [Figure 5.10].

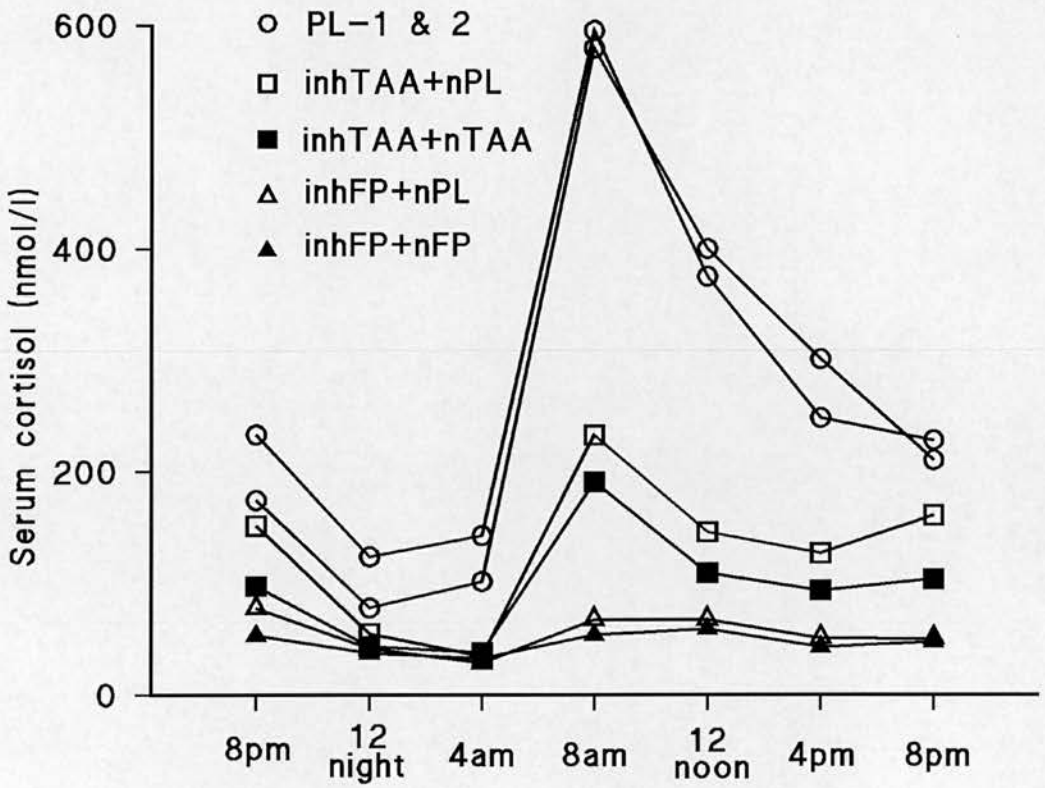


Figure 5.7
Geometric means for 24 hour profile of serum cortisol for first and second placebo (PL 1 and 2), inhaled triamcinolone acetonide with nasal placebo (inhTAA+nPL), inhaled fluticasone propionate with nasal placebo (inhFP+nPL), inhaled plus nasal triamcinolone acetonide (inhTAA+nTAA), inhaled plus nasal fluticasone propionate (inhFP+nFP).

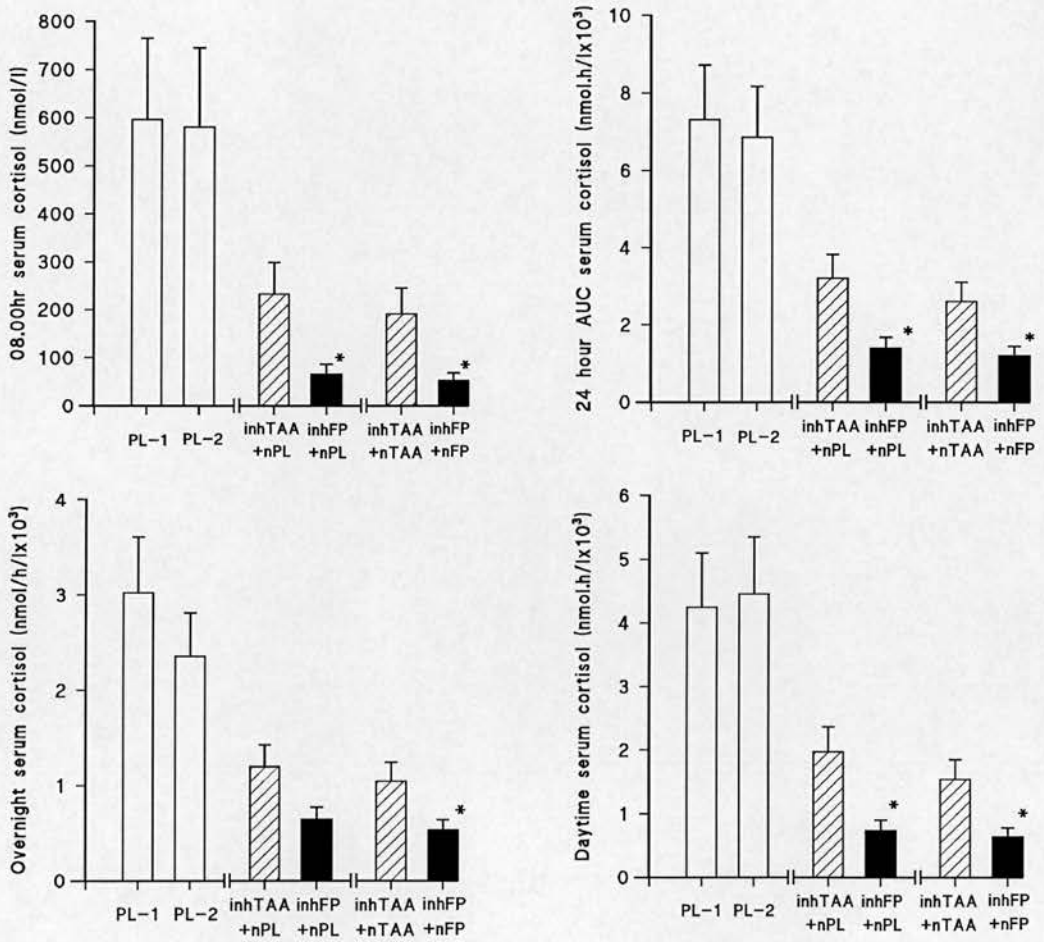


Figure 5.8
Geometric means with standard error for 24 hour and fractionated serum cortisol for first and second placebo (PL 1 and 2), inhaled triamcinolone acetonide with nasal placebo (inhTAA+nPL), inhaled fluticasone propionate with nasal placebo (inhFP+nPL), inhaled plus nasal triamcinolone acetonide (inhTAA+nTAA), inhaled plus nasal fluticasone propionate (inhFP+nFP). There was a significant($p<0.05$) difference between all 4 active treatments and both placebos. Asterisk denotes a significant ($p<0.05$) difference between FP and TAA for inhaled alone or inhaled plus nasal medication.

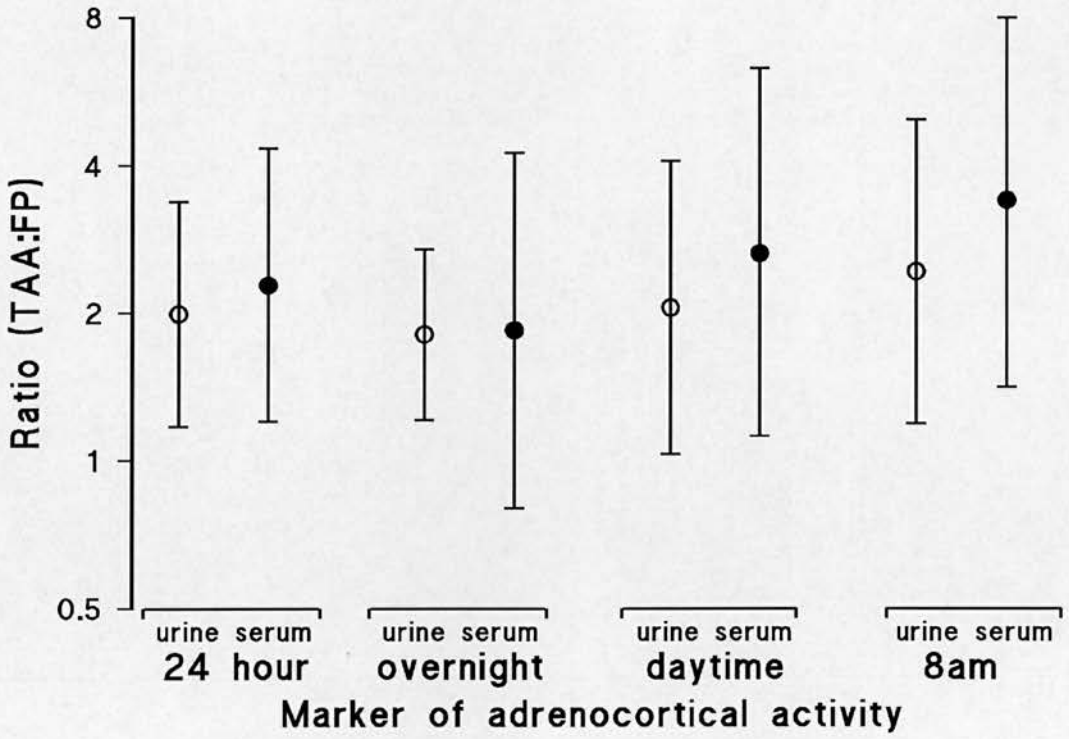


Figure 5.9
Ratio for TAA:FP (with 95% confidence intervals) for 24 hour, overnight, daytime and 8am urinary cortisol/creatinine excretion (open) & serum cortisol (closed). 95% confidence intervals which exclude unity indicate a significant difference between the two drugs.

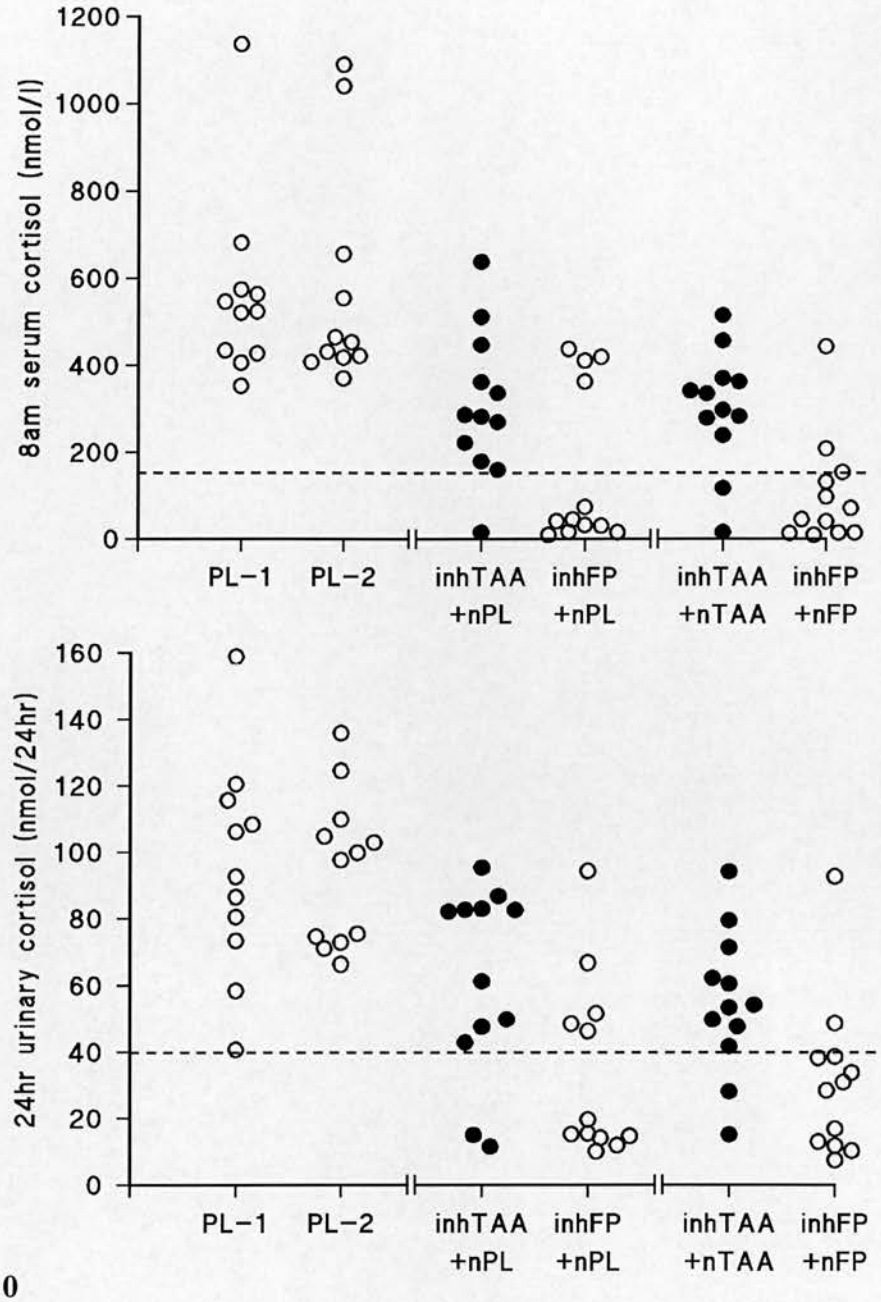


Figure 5.10

Individual values for a) 8am serum cortisol and b) 24 hour urinary cortisol for first and second placebo (PL 1 and 2), inhaled triamcinolone acetonide with nasal placebo (inhTAA+nPL), inhaled fluticasone propionate with nasal placebo (inhFP+nPL), inhaled plus nasal triamcinolone acetonide (inhTAA+nTAA), inhaled plus nasal fluticasone propionate (inhFP+nFP). For all 4 active treatments the numbers of individual patients with an abnormal 8am serum cortisol <150nmol/l (<5.4μg/dl) were: FP 17/24 (71%), and TAA 3/24 (12%) ($p<0.0005$). The corresponding numbers of individual patients with an abnormal 24 hour urinary cortisol excretion <40 nmol (<14.4μg) were FP 17/24 (71%) and TAA 4/24 (16%) ($p<0.0005$).

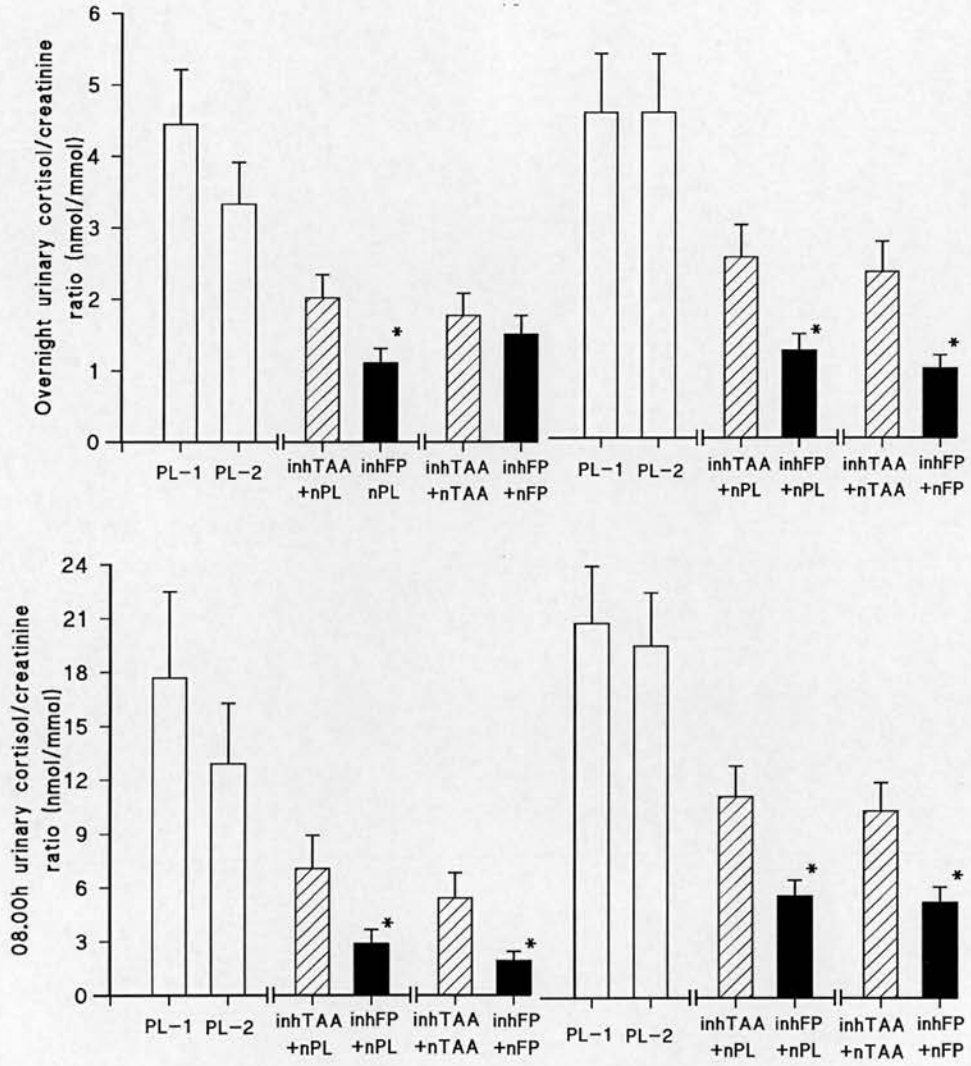


Figure 5.11
Geometric means with standard error for 24 hour corrected urinary cortisol/creatinine excretion and fractionated (overnight, 8am and daytime) corrected urinary cortisol/creatinine for first and second placebo (PL 1 and 2), inhaled triamcinolone acetonide with nasal placebo (inhTAA+nPL), inhaled fluticasone propionate with nasal placebo (inhFP+nPL), inhaled plus nasal triamcinolone acetonide (inhTAA+nTAA), inhaled plus nasal fluticasone propionate (inhFP+nFP). Asterisk denotes significant ($p<0.05$) difference between TAA and FP for inhaled or inhaled alone plus nasal medication. There was a significant difference between all 4 active treatments and both placebos for 24 hour and fractionated measurements.

5.4 DISCUSSION

In the first study, suppression of overnight urinary cortisol occurred with fluticasone propionate, triamcinolone acetonide and beclomethasone dipropionate, although this was only statistically significant for fluticasone propionate. The presence of detectable adrenal suppression does not necessarily imply that the observed effects are clinically relevant, although it is evident from the scatter plot (Figure 5.1b) that individuals differ in their susceptibility to the systemic adverse effects of intra-nasal corticosteroids. It is important to point out that this degree of suppression would not result in an acute adrenal crisis if patients were to abruptly stop their treatment or be exposed to acute stress. Indeed, there was no blunting of the cortisol response for any of the three drugs to low dose (0.5μg) ACTH stimulation test.

Although steady-state drug levels would have been reached in this study, it is possible that an impaired response to ACTH stimulation might have occurred after more prolonged treatment as a consequence of impaired adrenal reserve due to adrenocortical atrophy. However, previous long-term studies with clinically recommended doses of inhaled FP, TAA and BDP have shown no evidence of HPA-axis suppression in terms of a bolus or 6 hour infusion with high dose (250μg) ACTH[238,239,241].

The results with suppression of overnight urinary cortisol are in keeping with other studies which have shown systemic bioactivity with intra-nasal fluticasone propionate in terms of significant effects on serum osteocalcin[237]. The fact that intra-nasal

corticosteroids cause detectable systemic activity is not surprising as there is no first-pass inactivation in the nose, resulting in extensive systemic absorption of unchanged active drug[175]. Whether beclomethasone dipropionate undergoes partial biotransformation to active and inactive metabolites in the nose, as in the lung, is not clear.

These results are in contrast to a study by Vargas et al[340] where 200μg fluticasone propionate resulted in no significant change in morning plasma cortisol. In the same study fluticasone propionate 400μg bid resulted in significant plasma cortisol suppression but no blunting of the 250μg ACTH stimulation test. Studies have shown no difference from placebo in terms of plasma cortisol levels after 2 weeks[341], 4 weeks[139,342] and 1 year[342,343] of therapy with fluticasone propionate. Van As et al[241] performed a dose tolerance study of fluticasone propionate and showed no suppression of plasma cortisol or 24 hour urinary cortisol up to 1600μg per day in patients with seasonal allergic rhinitis. The study in this chapter investigated healthy volunteers and it is likely that nasal deposition and bioavailability might be altered in the presence of rhinitis, due to effects of inflammation and associated secretions.

Haye et al[344] performed a study comparing intra-nasal fluticasone propionate and beclomethasone dipropionate and showed that fluticasone propionate provided greater control of patients nasal blockage, discharge and eye watering after 1 year of therapy in patients with perennial allergic rhinitis. However, there was no significant difference

between the treatments in terms of morning plasma cortisol levels.

In the second study, the results showed no significant systemic bioactivity in terms of markers of adrenal function, blood count and bone metabolism in patients with allergic rhinitis. In particular, no suppressive effects were seen with the use of the sensitive measurement of 24 hour integrated plasma cortisol. Because the study was powered to detect 20% adrenal suppression, a smaller degree of suppression may not have been detected although this is unlikely to be of clinical relevance. Regardless, there were no trends to suggest that an increase in sample size would have shown a significant effect in any of the sensitive endpoints used. Great care was taken to ensure adequate nasal spray technique and compliance was checked with each patient at each visit and the 24 hour cortisol samples were taken in controlled conditions.

The findings of both studies, in healthy volunteers and patients with rhinitis, are in keeping with each other as neither showed significant suppression with triamcinolone acetonide. Studies have reported no significant suppressive effect with budesonide at a dose of 400μg per day, in terms of early morning plasma cortisol in adults with vasomotor rhinitis[345] and overnight urinary cortisol in children with seasonal allergic rhinitis[346]. In terms of blood eosinophil count, Edsbacker et al[347] showed detectable but clinically non-significant suppression with budesonide at a dose of 100μg. Knutsson et al[237] demonstrated significant suppression with budesonide in terms of osteocalcin, early morning serum cortisol and 24 hour urinary cortisol but this was at higher doses of

400μg od and 400μg bid administered to healthy volunteers. However, in the same study it was shown that there was no impaired response to dynamic stimulation with an insulin tolerance test. Pipkorn et al[345] also showed no impaired response to budesonide 400μg od in terms of the 6 hour cosyntropin test. In contrast two studies in children have shown evidence of growth suppression with intra-nasal budesonide pMDI or dry powder inhaler 400μg per day, as assessed by knemometry[348,349].

These findings with mometasone furoate are also in keeping with those of Brannan et al who found, in two separate studies, no suppression with once daily intra-nasal mometasone furoate at doses of 200μg and 400μg in adults[238], and 50 to 200μg in children[350]. Although 24 hour urinary free cortisol was investigated in children, the main endpoint for adrenal suppression in both studies was the 6 hour 250μg cosyntropin stimulation test.

Mometasone furoate is more potent than both budesonide and triamcinolone as shown by in vivo inhibition of T-cell cytokine production[351]. It is difficult to explain its lack of systemic bioactivity in terms of glucocorticoid potency, first-pass metabolism or in terms of sensitivity of tissue markers. The explanation may be due to the fact that all of the corticosteroids studied had short plasma elimination half lives (BUD 2.3 hrs, TAA 3.6 hrs, MF 5.8 hrs)[159,165] and therefore at steady-state with once daily dosing, significant degree of blood accumulation is unlikely to occur. It is known that adrenal suppression, unlike clinical efficacy, is proportional to plasma concentration. In this

respect, the adrenocortical suppression with fluticasone propionate, which has a longer elimination half life (14.4hrs)[158], is greater with steady-state than single dosing[316] and fluticasone propionate has been shown to cause more suppression than other corticosteroids when given by the intra-nasal (Chapter 8) or inhaled route (Chapter 3)[231]. Another possibility for the lack of effects is that the aqueous delivery device may be less efficient for MF resulting in a lower degree of nasal bioavailability, although there are no published data for Nasonex nasal formulation. Deposition data using positron emission tomography delivery scanning showed that the thixotropic formation of triamcinolone acetonide produced 80% delivery to the target tissues in the nose and sinuses[338].

When assessing the combined effects of intra-nasal corticosteroids and inhaled corticosteroids, it was shown for 24 hour and fractionated measurements of serum cortisol and corrected urinary cortisol/creatinine excretion, that both inhaled fluticasone propionate and triamcinolone acetonide produced significant adrenal suppression versus placebo. Inspection of the 24 hour serum cortisol profile for fluticasone propionate shows that the normal circadian diurnal rhythm was abolished. This flattening of the 24 hour profile with fluticasone propionate has also been reported by Boorsma[183] in healthy subjects taking 1000μg bid of fluticasone propionate (pMDI without spacer) with 87% suppression in AUC for plasma cortisol. Interestingly in the asthmatic patients in this study the degree of suppression for AUC was calculated at 81% with inhaled fluticasone propionate (pMDI without spacer) at a dose of 880μg bid. Although

triamcinolone acetonide showed significant suppression, the diurnal pattern remained intact with a cortisol rise at 8am.

From the 24 hour serum profile, it can be seen that the addition of intra-nasal to inhaled medication had a small non-significant additive effect with triamcinolone acetonide. With fluticasone propionate, however, there was no evidence of any additional suppression with intra-nasal corticosteroids which can be explained by the near maximal suppression with the inhaled drug alone. In other words the ceiling of the dose response curve had been attained with inhaled fluticasone propionate alone prior to addition of intra-nasal fluticasone propionate. It is evident from inspection of the scatter-plots for 8am serum cortisol and 24 hour urinary cortisol excretion that the majority of patients receiving inhaled fluticasone propionate alone or with intranasal fluticasone propionate, had suppression below that of accepted normality[176]. In contrast there were only a minority of abnormal individual low values for inhaled alone or with intranasal triamcinolone acetonide. Although the mean data showed no significant additive suppression with intranasal fluticasone propionate, it was evident from the individual data for 24 hour urinary cortisol that there were a further three abnormal values with combination therapy as compared to inhaled fluticasone propionate alone. This in turn suggests that the bioavailability from the nasal moiety may contribute to the overall systemic burden in certain susceptible patients[175].

CHAPTER 6

DOSE RESPONSE EVALUATATION OF THE THERAPEUTIC INDEX FOR INHALED BUDESONIDE IN ASTHMATIC ADULTS

6.1 INTRODUCTION

The preceding chapters have assessed the dose-response effect for systemic bioactivity and adverse effects of inhaled and intra-nasal corticosteroids. They have shown that inhaled corticosteroids exhibit dose-related systemic adverse effects. Furthermore, intra-nasal corticosteroids may also exhibit systemic adverse effects in some patients and this is especially the case when given in addition to inhaled corticosteroids. However, the unwanted systemic side effects represent only half of the picture, and it is necessary to consider the therapeutic benefit of these drugs. This is required in order to calculate a therapeutic ratio. In this respect, it is clear that the therapeutic ratio is of greater clinical importance as this represents the trade off between harmful and beneficial effects of a drug.

Inhaled corticosteroids are widely accepted as first-line anti-inflammatory therapy for the treatment of persistent asthma[352]. Current asthma management guidelines suggest that the dose of inhaled corticosteroids should be titrated according to the patients' symptoms or lung function[134,353]. However, measurements of lung function reflect the consequences of the inflammatory cascade rather than quantifying the degree of underlying inflammation per se. In other words it is theoretically possible for patients to have bronchodilation, for example with inhaled β_2-adrenoceptor agonists, but still have uncontrolled airways inflammation. There is concern that this may result in a delay in treatment of an asthmatic exacerbation[272].

As discussed above (see section 1.2), there are a number of techniques for assessing asthmatic disease. These include patients' symptoms and spirometry. However the degree of underlying airway inflammation can be assessed non-invasively by quantifying bronchial hyperresponsiveness[84]. Methacholine bronchial challenge, which correlates with airway eosinophil numbers[76], is thought to be less clinically relevant than adenosine monophosphate, which more closely mimics bronchial hyperreactivity due to mast cell activation and inflammatory mediator release[79]. However, there are no data comparing these types of challenge in a dose-ranging study with inhaled corticosteroids.

Exhaled nitric oxide production is induced by inflammatory cytokines and therefore may also be a marker of underlying airway inflammation[94]. Both peripheral blood eosinophil count and their state of activity, as measured by serum ECP concentration, are also considered to be sensitive surrogate markers of asthmatic inflammation[108,112]. However, as all of the current available markers of asthmatic disease control assess different parts of the inflammatory cascade they should not be considered in isolation but must be considered together.

It is also recognised that inhaled corticosteroids cause dose-related systemic effects on tissues such as the adrenal gland and bone. In particular, sensitive measures of basal adrenocortical activity such as overnight urinary cortisol excretion may be used to detect potential systemic bioactivity of inhaled corticosteroids[175]. This study is therefore a dose-ranging evaluation of an inhaled corticosteroid on symptoms, lung function,

markers of airway inflammation as well as systemic adverse effects.

Budesonide was used as an example of a commonly used inhaled corticosteroid which could be administered by breath actuated dry powder reservoir device in order to improve lung delivery and compliance. The doses were chosen to reflect the clinically recommended dose range (400-1600μg per day) for this drug. Kraan et al[251] showed that two weeks of treatment with inhaled budesonide was adequate to achieve near maximal response in terms of effects on bronchial challenge and lung function. Hence the medication was administered for 3 weeks at each dose sequentially over a total period of 9 weeks.

6.2 METHODS

Patients

Twenty-six (13 female) mild to moderate asthmatic patients were recruited into the study, mean (SE) age 34.7 (2.3) years, FEV_1 84.8 (3.0) % predicted, FEV_{25-75} 55.5 (3.4) % predicted. All patients were taking maintenance inhaled corticosteroids (median dose 800μg per day; range 200-1600μg per day) (beclomethasone n=19, budesonide n=6, fluticasone n=1). All patients were required to be responsive at screening to methacholine and adenosine monophosphate challenge testing with a provocation dose/concentration producing 20% fall in FEV_1 of less than 500μg (geometric mean PD_{20} MCh 32.8μg) and 200mg/ml (geometric mean PC_{20} AMP 35.8mg/ml) respectively. Two patients were taking oral theophylline therapy and two patients were taking long acting β_2 therapy prior to entry into the initial placebo run-in. All but one patient were shown to be atopic by Phadiotop testing.

Design

The study was conducted as an open label study. Patients had an initial placebo run-in period of 10 days where they received a placebo Turbuhaler one puff twice daily at 8am and 8pm. Following this patients received budesonide as Pulmicort Turbuhaler 200μg per actuation (Astra Pharmaceuticals, Kings Langley, UK) in three consecutive doubling dose increments each of 3 weeks (i.e. a total of 9 weeks): 1 puff bid, 2 puffs bid, 4 puffs bid at 8am and 8pm:- i.e. a total daily dose of 400μg, 800μg, and 1600μg. Patients attended the laboratory after the run-in placebo period (at baseline) and after each 3

week period of inhaled budesonide. After 3 weeks of high dose inhaled budesonide patients returned to the laboratory, on the day following the fourth visit, for a fifth occasion when spirometry and bronchial challenge testing were repeated 20 minutes after inhalation of a bolus dose of 400μg inhaled salbutamol (Ventolin Accuhaler, Allen & Hanburys Ltd., Uxbridge, UK).

Patients withheld all other treatment with long-acting β_2-agonists, cromones, theophylline or leukotriene antagonists throughout the study. Patients received their usual short acting β_2 agonist on an as required basis but were asked to withhold this for 12 hours prior to attending the laboratory.

Measurements

The following measurements were made after the placebo period and after each three week treatment block:

8am plasma cortisol

8am serum osteocalcin

8am serum eosinophilic cationic protein

8am peripheral blood eosinophil count

Overnight urinary cortisol excretion

Adenosine monophosphate bronchial challenge

Methacholine bronchial challenge

Exhaled nitric oxide

Spirometry

Domiciliary symptom score

Domiciliary twice daily peak expiratory flow rate

Also in a subgroup of 15 patients:

hCRF stimulation test

Statistical Analysis

The study was designed with at least 80% power to detect a 1.0 doubling dose difference (2.0 fold) in AMP PC_{20} and MCh PD_{20}[354], with the alpha error set at 0.05 (two-tailed).

For domiciliary peak expiratory flow the mean values of the last 7 days of each treatment period were analysed, whereas for asthma symptom scores the sum of the last 7 days of each treatment period were used.

Overall comparisons between treatment levels were made by multifactorial analysis of variance (MANOVA) using subject and treatment as factors. This was followed by Bonferroni multiple range testing (set at 95% CI) to assess which doses were different from baseline, in order to obviate multiple pair-wise comparisons. Consequently statistical comparisons are only denoted as being significant ($p<0.05$) or not significant in order not to confound the alpha error. Regression analysis was applied to investigate whether for each endpoint there was a significant overall dose-response relationship for all three doses of budesonide. In order to permit quantitative comparisons between the

different endpoints, the response after placebo run-in and after each dose level was calculated as the percentage of maximal achievable response occurring at the highest dose level (1600μg per day).

6.3 RESULTS

Anti-asthmatic efficacy

There was a significant difference between placebo compared with medium and high doses of budesonide for mean spirometry values (FEV_1, FEF_{25-75}, and PEF). The mean percentage change from baseline was greater for FEF_{25-75} than for PEF or FEV_1 at all dose levels [Figure 6.1]. For AMP and MCh challenge there were significant overall dose response effects and all doses of budesonide were significantly different from placebo [Table 6.1]. There was a greater doubling dose shift for AMP than for MCh challenge across the dose range [Figure 6.2]. A 400μg bolus dose of inhaled salbutamol produced further improvements in both MCh, AMP and lung function over and above the response with budesonide 1600μg per day [Table 6.1, Figure 6.1, Figure 6.2]. Differences from placebo were significant at all 3 doses of budesonide for nitric oxide and diary card data, but only at the medium and high doses for eosinophil markers.

When expressed as a percentage of maximal response [Figure 6.3], there was a plateau in the dose-response curve for nitric oxide at 400μg per day, for spirometry at between 400μg per day and 800μg per day and for ECP and AMP at between 800μg per day and 1600mg per day.

Systemic effects

Markers of HPA-axis activity showed a significant overall dose-response relationship, with 8am plasma cortisol exhibiting no plateau in response within the evaluated dose-

range[Table 6.2]. The proportion of individuals with a stimulated plasma cortisol response (post hCRF) less than 500nmol/l were: baseline (7%); 400μg per day (13%), 800μg per day (40%), 1600μg per day (66%) and for overnight urinary cortisol less than 20nmol/10hr were: baseline (15%); 400μg per day (12%); 800μg per day (42%); 1600μg per day (43%). With osteocalcin there was no evidence of a significant dose-response relationship with none of the doses being statistically significant from placebo [Table 6.2].

Therapeutic index

To evaluate the therapeutic index (airway/systemic ratio) patients were categorised into those who did (overnight urinary cortisol <20nmol/10hr) or did not (overnight urinary cortisol >20nmol/10hr) have a marked systemic response and/or those who did (AMP > 2 doubling dose shift) or did not (AMP <2 doubling dose shift) have a marked airway anti-inflammatory response with inhaled budesonide [Figure 6.4]. The proportion of patients with a marked airway response together with a minimal systemic response was 46% at low dose and 52% at high dose. The proportion of patients with a marked airway response together with a marked systemic response increased from 4% at low dose to 38% at high dose.

Table 6.1

Mean (SE) for forced expiratory volume in 1 sec (FEV_1), forced mid-expiratory flow rate (FEF_{25-75}), peak expiratory flow rate (PEF) as percentage predicted (%pred); and for adenosine monophosphate (AMP) bronchial challenge test as provocative concentration causing a 20% fall in FEV_1 (PC_{20}) and methacholine (MCh) bronchial challenge testing as provocative dose causing a 20% fall in FEV_1 (PD_{20}). Values are shown after placebo (baseline), 400μg per day, 800mg per day, 1600μg per day of inhaled budesonide and following a bolus dose of 400μg inhaled salbutamol. The 'p' values for the significance of the slope of the overall dose response curve are also shown. An asterisk denotes a significant difference compared to placebo run-in (baseline). Values for AMP and MCh are geometric means.

Visit	FEV_1 % pred	FEF_{25-75} % pred	PEF % pred	AMP PC_{20}	MCh PD_{20}
	%	%	%	mg/ml	μg
Baseline	78.0 (3.2)	53.1 (3.5)	89.3 (3.8)	23.7 (7.4)	17.7 (5.4)
400μg	81.4 (3.1)	56.5 (3.0)	92.9 (3.1)	133.8 * (40.2)	48.8 * (14.3)
800μg	84.9 * (3.0)	61.9 * (3.8)	96.8 * (2.7)	300.8 * (92.0)	58.2 * (16.1)
1600μg	84.5 * (3.1)	59.3 * (3.3)	97.5 * (3.3)	502.2 * (146.3)	119.3 * (44.3)
Post salbutamol	89.5 * (3.3)	66.1 * (4.3)	100.7 * (3.6)	1322.8 * (227.1)	525.0 * (144.9)
p value for dose-response	0.106	0.123	0.052	<0.001	<0.001

Table 6.2

Mean (SE) for exhaled nitric oxide (NO), blood eosinophil count (EOS), serum eosinophilic cationic protein (ECP), morning domiciliary peak expiratory flow rate (PEFam), evening domiciliary peak expiratory flow rate (PEFpm), asthma symptom scores (Symp), 8am plasma cortisol (8am cortisol), overnight urinary cortisol (OUC), and osteocalcin (O'cal) after placebo (baseline), 400μg per day, 800μg per day and 1600μg per day of inhaled budesonide. The 'p' values for the significance of the slope of the overall dose response curve are also shown. Asterisk denotes a significant difference compared to placebo run-in (baseline).

	NO	EOS	ECP	PEF am	PEF pm	Symp	8am cortisol	OUC	O'cal
	ppb	$x10^9$/l	nmol/l	l/min	l/min	units	nmol/l	nmol/ 10hr	nmol/l
baseline	19.1 (2.2)	0.41 (0.05)	20.1 (3.5)	390.7 (16.9)	410.0 (19.1)	13.2 (1.4)	528.9 (39.0)	61.1 (9.5)	0.97 (0.11)
400μg	9.2 * (0.9)	0.35 (0.04)	15.9 (1.7)	433.4 * (17.8)	440.8 * (18.1)	7.3 * (1.7)	528.6 (36.1)	40.0 (4.4)	0.92 (0.12)
800μg	8.8 * (1.0)	0.31 * (0.04)	12.8 * (2.0)	443.8 * (16.0)	455.2 * (15.7)	6.6 * (1.9)	461.4 (38.2)	28.4 * (3.1)	0.85 (0.12)
1600μg	7.2 * (0.6)	0.26 * (0.03)	10.2 * (1.2)	461.3 * (17.0)	506.7 * (45.0)	3.4 * (1.1)	366.9 * (36.1)	24.9 * (3.2)	0.87 (0.10)
p value for dose-response	<0.001	<0.005	<0.005	<0.01	<0.05	<0.001	<0.005	<0.001	0.458

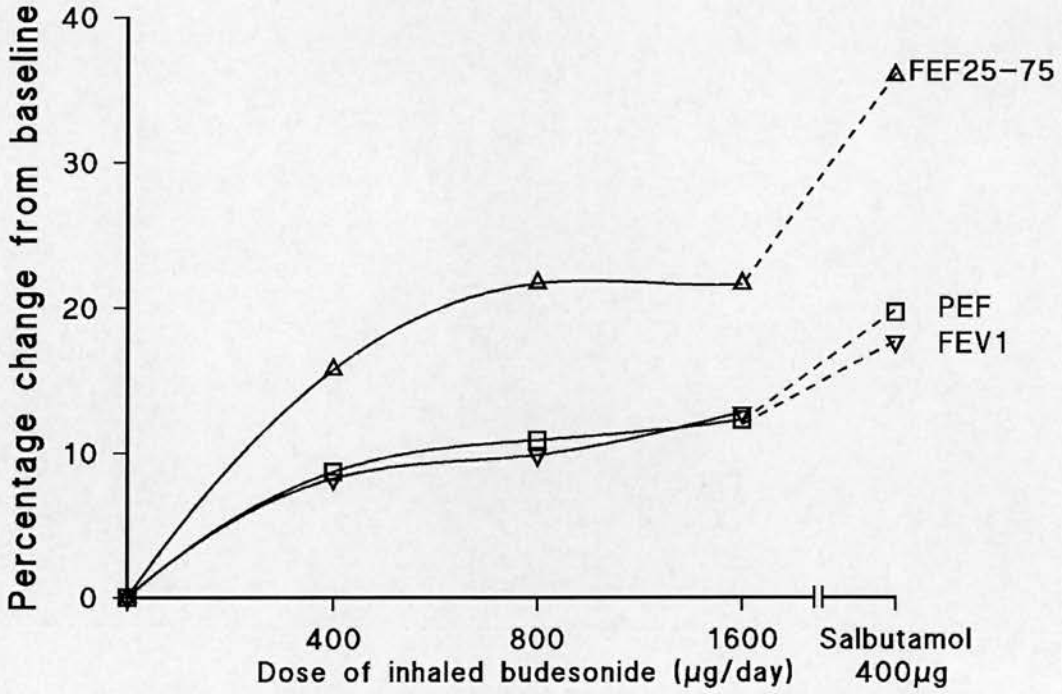


Figure 6.1
Percentage change from placebo run-in (baseline) for laboratory based spirometry data (FEV_1,FEF_{25-75},PEF) after three weeks each at low, medium and high doses of budesonide and after bolus 400µg dose of inhaled salbutamol (i.e. at the end of the 1600µg per day budesonide period). There was a significantly greater percentage change for FEF_{25-75} than for FEV_1 or PEF responses.

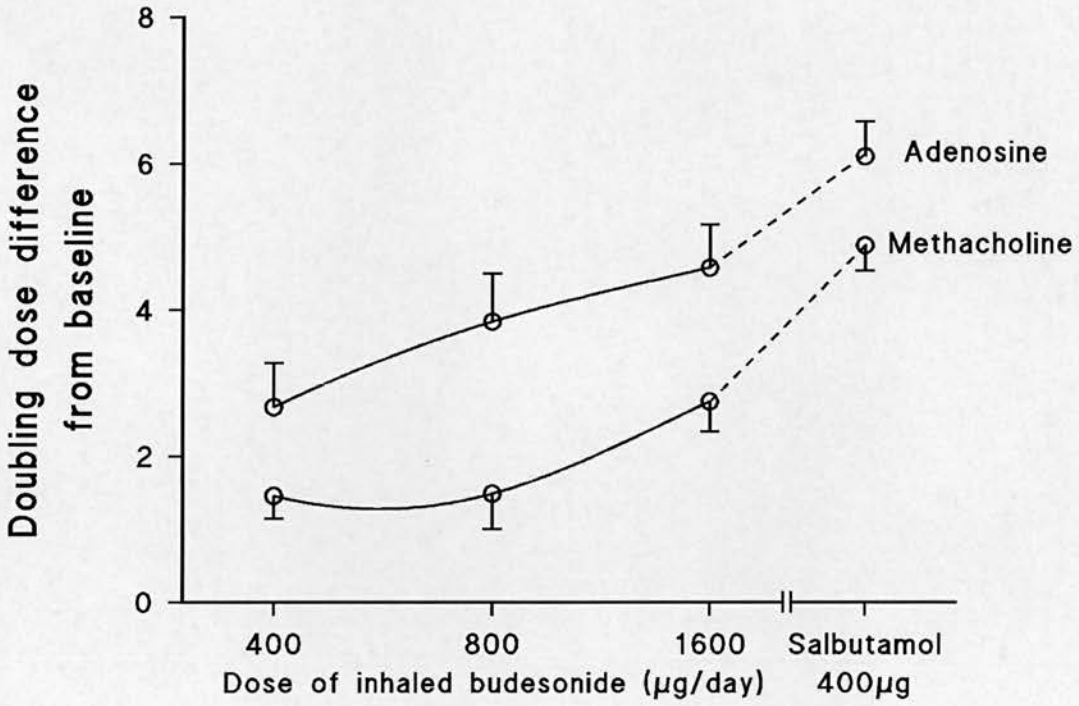


Figure 6.2
Doubling dose shift from placebo run-in (baseline) for adenosine monophosphate and methacholine bronchial challenge testing at each dose of budesonide and after a bolus of salbutamol. There was a significantly shift with adenosine than methacholine which was approximately parallel across the dose range.

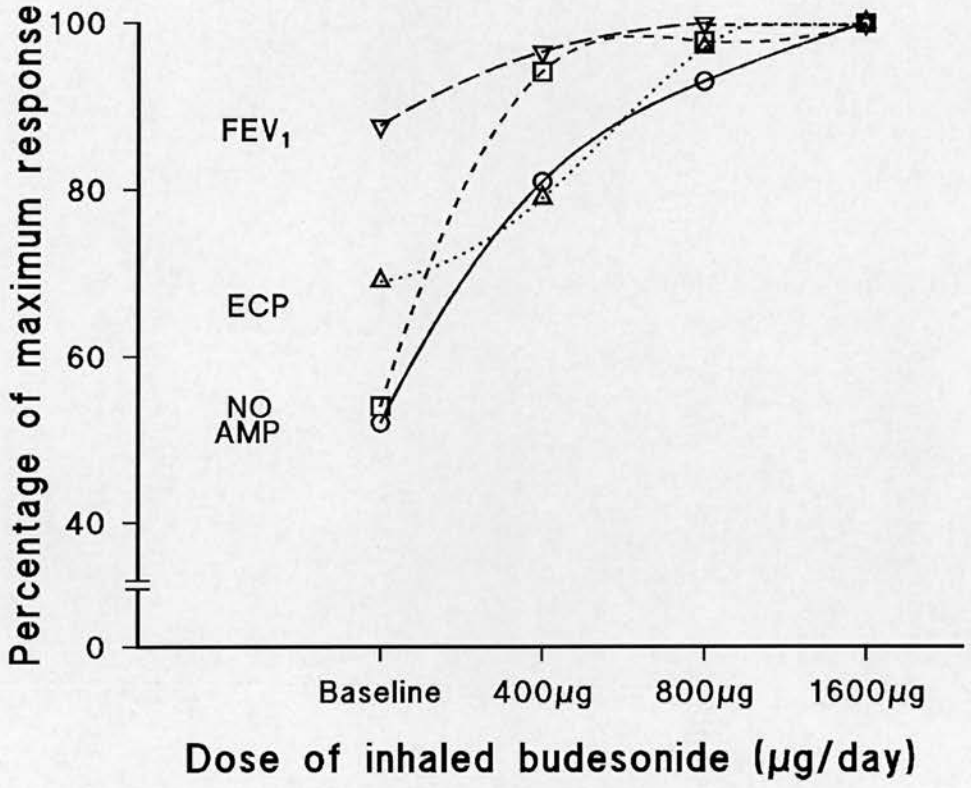
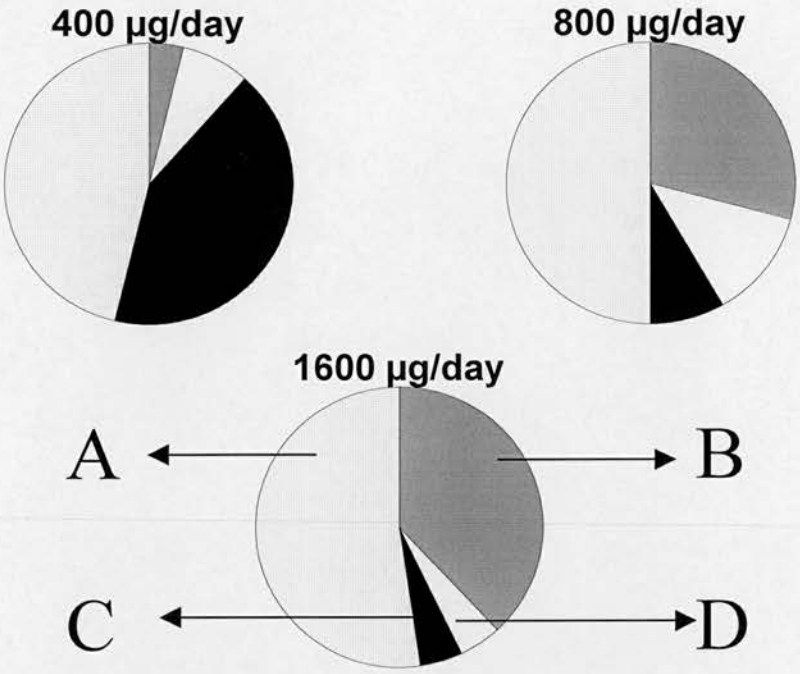


Figure 6.3
Anti-asthmatic efficacy parameters plotted together as percentage of maximum achievable response after placebo (at baseline), 400µg per day, 800µg per day, 1600µg per day of inhaled budesonide for FEV_1 (∇), serum eosinophilic cationic protein (ECP: Δ), exhaled nitric oxide (NO: □) and adenosine monophosphate bronchial challenge (AMP: O).



		Low dose 400µg per day	Medium dose 800µg per day	High dose 1600µg per day
A	Overnight urinary cortisol>20nmol, AMP>2 doubling concentrations	46%	50%	52%
B	Overnight urinary cortisol <20nmol, AMP>2 doubling concentrations	4%	29%	38%
C	Overnight urinary cortisol <20nmol, AMP<2 doubling concentrations	8%	13%	5%
D	Overnight urinary cortisol >20nmol, AMP<2 doubling concentrations	42%	8%	5%

Figure 6.4
Pie charts for proportion of individual patients at each dose level with A) overnight urinary cortisol (OUC) > 20nmol/l and adenosine monophosphate bronchial (AMP) challenge > 2 doubling doses (dd) B) OUC <20 nmol and AMP > 2 dd C) OUC <20 nmol and AMP <2dd black) D) OUC >20nmol and AMP <2 dd.

6.4 DISCUSSION

The results from this study indicate that, in patients with mild to moderate atopic asthma, the mean responses for lung function parameters were optimised at a dose of 400μg - 800μg per day of inhaled budesonide. For parameters of asthmatic inflammation, including serum eosinophil cationic protein and bronchial hyperreactivity to methacholine and adenosine monophosphate bronchial challenge, maximal effects were obtained at a dose of 800mg - 1600μg per day. The relevance of these findings is that the underlying inflammation may be inadequately controlled if the dose of inhaled budesonide is titrated according to the patients' lung function.

It should be noted that with nitric oxide, in contrast to other markers of inflammation, there was a plateau in response at 400μg per day. This is in keeping with Jatakanon et al[355] who showed that exhaled nitric oxide was normalised at 400μg per day. However, this may reflect the sensitivity of nitric oxide to inhaled corticosteroids (see section 1.2.3) rather than absolute control of inflammation and highlights the importance of assessing more than one marker of inflammation[356].

Inhaled corticosteroids have been shown to exhibit dose-related systemic bioactivity which has lead to concern regarding the potential long term adverse effects of high dose therapy (Chapters 3 and 4). In the present study, there was dose related suppression of adrenocortical activity, as assessed by mean values for overnight urinary cortisol or plasma cortisol pre and post hCRF stimulation. However, it is more important to

consider the effects of increasing the dose of inhaled corticosteroid on the overall therapeutic index, rather than adverse or beneficial effects in isolation. In this respect, the proportion of individuals with a good airway anti-inflammatory response (an AMP challenge greater than 2 doubling doses) at high dose was 90%, compared to 50% at low dose although this was offset by greater adrenal suppression at the high dose (38%).

Unfortunately as only one drug was investigated, the study had to be of an open label design. However, when performing the investigations measuring clinical response, for example the bronchial challenge tests or nitric oxide measurements, it was not known which dosing schedule the patient was on as the examiner did not refer back to the results of previous visits.

This study was of an escalating dose design. In other words, the patients received doubling doses of drug at each limb of the study without a washout period. As discussed elsewhere (see section 10.6) this may result in a time-effect as well as a dose-effect on treatment response. Ideally the order of the dose-steps should have been randomised and there should have been a washout period in between each dose. However, this would have significantly lengthened the duration of the study and increased the patient drop-out rate.

Another weakness of this design is the way in which the doses were given. The study was designed so that the concentration of each inhaler remained constant and patients took 1 puff twice daily followed by 2 puffs twice daily then 4 puffs twice daily. This

may have altered patient compliance and they could have expected their symptoms to improve if they were under the impression that they were receiving increasing doses of the drug. It would have been better to have taken two puffs twice daily throughout the study and use inhalers of 100μg, 200μg and 400μg per actuation.

The duration of each treatment period was also relatively short. As is discussed elsewhere (see section 10.11), it is possible that there may have been a greater response to bronchial challenge testing had the duration of each treatment period be longer. However, the effect of dose is likely to have contributed to a greater extent than the effect of duration of treatment on the bronchoprotector response to methacholine.

The results of this study are in keeping with those of Busse et al[357] in a parallel study comparing 200μg, 400μg 800μg and 1600μg per day of inhaled budesonide via a Turbuhaler. After 12 weeks of therapy there was no significant difference between the response of 400μg per day and 1600μg per day in terms of change in mean in FEV_1 from baseline, indicating a plateau in response. There was also no dose-response effect in terms of symptom control, with all doses being statistically superior to placebo but no significant difference between doses. In another study with triamcinolone acetonide via a spacer, in mild to moderate asthmatic patients, there was also a plateau in response for symptoms and lung function at 400μg per day[358].

The results for bronchial hyperreactivity are similar to those of Kraan et al[251] who

showed a significant difference in terms of methacholine bronchial challenge between 200μg per day and 800μg per day of inhaled budesonide in a two-way parallel study. Measurements were made after 2, 4, 6 and 8 weeks and the difference between these doses remained significant throughout the study. The dose-response effect of budesonide Turbuhaler has also been reported by Jatakanon et al[355] in mild asthmatics although their data was a composite of two separate parallel group studies in patients receiving 4 weeks with either 100μg per day (n=8), 400μg per day (n=7) or 1600μg per day (n=10). They found a dose-response effect for methacholine bronchial challenge PC_{20} and induced sputum eosinophil cell counts with no evidence of plateau.

More recently, Taylor et al[359] showed a dose-response effect with 100μg, 400μg and 1600μg per day of inhaled ciclesonide in terms of adenosine monophosphate bronchial challenge testing but not in terms of induced sputum ECP levels. Pedersen et al[247] showed a dose response effect between 100 and 400μg per day of budesonide via a spacer for the effects on exercise protection, but there were no improvements in symptoms, domiciliary peak expiratory flow or β2-agonists use with doses greater than 100μg per day.

When comparing the effects of adenosine monophosphate and methacholine bronchial challenge test, the results of this study indicate that adenosine monophosphate is more sensitive in terms of changes with inhaled corticosteroids and with inhaled β_2 agonists. This is in keeping with a study by O'Connor et al[360], who have also shown greater

effect of inhaled budesonide on adenosine 5'-monophosphate than on sodium-metabisulfite bronchial challenge in patients with mild asthma. Furthermore, Egbagbe et al[361] showed terbutaline to have a greater effect on adenosine monophosphate than histamine bronchial challenge, which is also in keeping with the results of this study.

It has been suggested that unchecked long-term inflammation leads to the development of chronic irreversible air-flow limitation[261]. Patients with mild-intermittent asthma have evidence of ongoing airway inflammation and may show improvement in inflammatory markers when receiving inhaled corticosteroids[362]. Studies in children[363] and in adults[364,365] have shown that a delay in instituting treatment with inhaled corticosteroids may result in an attenuated response to inhaled steroid therapy. It is clear, therefore, that patients have a better outcome when inhaled corticosteroid treatment is commenced early in the course of the disease. For the same reasons, it is also possible that patients will also fare better if the dose of inhaled corticosteroid is sufficient to optimise suppression of airway inflammation.

Previous studies with inhaled corticosteroids and long-acting β_2-agonists are shown to have additive effects on lung function and exacerbation rates[62]. Further improvement in bronchial hyperreactivity were found with a β2-agonist on top of high dose inhaled budesonide. These effects of β2-agonists on bronchial hyperreactivity are due to functional antagonism on smooth muscle, although it is considered that effects on AMP challenge are also due to direct inhibition of mast cell β2 adrenoceptor[366].

In keeping with the steep dose response with spirometry and shallow for bronchial challenge, Noonan et al[248] found a significant increase with both fluticasone propionate 100μg per day and 200μg per day versus placebo, but no difference between the two doses. However, with methacholine challenge, there was significant improvement with fluticasone propionate 200mg per day between both lower dose fluticasone propionate and placebo, and there was no difference between fluticasone propionate 100μg per day and placebo. Therefore, the addition of methacholine challenge testing allowed definition of a dose-response relationship that was not apparent with spirometry.

CHAPTER 7

A COMPARISON OF TOPICAL BUDESONIDE AND ORAL MONTELUKAST IN SEASONAL ALLERGIC RHINITIS AND ASTHMA.

7.1 INTRODUCTION

It has been shown in the previous chapter, that patients may have underlying airway inflammation even when their symptoms are controlled and their lung function is normal. It was also suggested, however, that inhaled corticosteroids exhibit dose-related adverse systemic effects and indeed a high dose may confer a reduced therapeutic index. For this reason, alternatives to inhaled and intra-nasal corticosteroids need to be considered as a therapeutic option either to be used as monotherapy or as second-line therapy in conjunction with corticosteroids. The aim of these second-line anti-inflammatory therapies should be to control symptoms and improve quality of life. However, as asthma and allergic rhinitis are inflammatory conditions these treatments are also required to control airways inflammation which is thought to be the underlying mechanism producing the symptoms (see section 1.1)

Methods used to infer the degree of airway inflammation were exhaled and nasal nitric oxide and adenosine monophosphate bronchial challenge[79,94]. Domiciliary peak expiratory flow rate and peak inspiratory nasal flow were used, and patients recorded their asthma symptom and seasonal allergic rhinitis symptoms in terms of eye, throat and nasal symptoms, which is in keeping with international guidelines[14]. Nasal peak inspiratory flow rate has been shown to exhibit comparable results to rhinomanometry[367] and has recently been reported to correlate well to patients subjective measure of seasonal allergic rhinitis symptoms[65].

Topically delivered inhaled and intra-nasal corticosteroids are widely recognised to be effective anti-inflammatory treatment for allergic rhinitis and asthma[134,136]. Leukotriene receptor antagonists, however, have also been shown to have anti-inflammatory properties and to have beneficial effects on asthma disease control[286]. They have recently been licensed as monotherapy for use in mild asthma and as second line therapy for more severe asthma.

As both asthma and allergic rhinitis are mediated by similar inflammatory mechanisms[36], it is likely that leukotriene receptor antagonists will also have anti-inflammatory activity in allergic rhinitis[368]. Indeed there are data showing symptomatic benefit with such treatment[310,312]. Orally administered leukotriene antagonists act systemically and thus, in theory, both conditions may be treated simultaneously.

The aim of this study was to evaluate whether an oral leukotriene receptor antagonist would be efficacious in patients with seasonal allergic rhinitis and asthma, and also how it would compare to inhaled and intra-nasal corticosteroids. In keeping with most treatment regimens for allergic rhinitis, and to maximise compliance, treatment was administered once daily. Budesonide and oral montelukast were used as these are the only treatments in their class which are currently licensed for once daily administration in asthma.

7.2 METHODS

Patients

Twelve patients with seasonal allergic rhinitis and asthma (9 female), mean (SE) age 34.7 (2.3) years, FEV_1 84.8 (3.0) % predicted were recruited into the study. Six patients were taking inhaled corticosteroids (budesonide 400μg per day n=2; beclomethasone 500μg per day n=2, 400μg per day n=1, 200μg per day n=1), 3 were taking intra-nasal corticosteroids (beclomethasone 400μg per day) and 5 were taking oral antihistamines (loratadine 10mg per day n=3, cetirizine 10mg per day n=2). All patients were required to be responsive to adenosine monophosphate challenge testing with a provocation concentration producing 20% fall in FEV_1 of less than 200mg/ml (geometric mean 29mg/ml). All patients had a positive skin prick test to grass or tree pollens and 9 patients also had positive skin prick test to house dust mite.

Design

The study was of a randomised placebo controlled, single-blind, double dummy, cross-over design. Patients were recruited during June and July 1998 when grass and tree pollen levels are usually high in Tayside. Patients were randomised to receive the following all given once daily at 0800hrs: A) 400μg inhaled budesonide dry powder (as Pulmicort Turbuhaler™, Astra Pharmaceuticals Ltd., UK as 2 puffs of 200μg per actuation) plus 200μg intranasal aqueous budesonide (as Rhinocort Aqua™ , Astra Pharmaceuticals Ltd., UK as 1 squirt of 100μg in each nostril) plus placebo tablets; or B) 10mg oral montelukast (as Singulair™, Merck Sharp & Dohme Ltd., Herts, UK) plus

placebo nasal spray and placebo Turbuhaler. Prior to each treatment, and at cross over, patients had a one week treatment period with placebo Turbuhaler (2 inhalations), placebo nasal spray (2 squirts in each nostril) and placebo tablets all taken at 0800hrs. Six patients started with oral montelukast and 6 patients started with topical budesonide.

Measurements

The following measurements were made after both placebo and each active treatment periods:

Adenosine Monophosphate Challenge Testing

Exhaled and Nasal Nitric Oxide

Spirometry

And throughout the study:

Diary Card Data

Pollen Count Measurement

Statistical Analysis

The study was powered at the 80% level to detect a 1.0 doubling dose difference (2.0 fold) in adenosine monophosphate PC_{20} with the alpha error set at 0.05 (two-tailed). On each day a total seasonal allergic rhinitis symptom score was calculated as the sum of each patients' individual nose, eye and throat symptom scores. Daily tree, grass and weed pollen count data were summed to provide a daily pollen score for each day of the study. For all domiciliary diary and pollen data, mean values for the 7 day placebo periods and for the last 7 days of active treatment period were analysed. Overall

comparisons between active treatments and placebos were made by multifactorial analysis of variance (MANOVA) using subject, treatment and period as factors. Analysis of co-variance was used to account for any influence of pollen level. This was followed by Bonferroni multiple range testing (set at 95% CI) in order to obviate multiple pair-wise comparisons. Consequently comparisons are only denoted as being significant ($p < 0.05$) or not significant in order to not confound the alpha error. Least squares regression analysis was used to assess the correlation between morning and evening nasal PIFR with nasal symptom scores, and morning and evening PEF with asthma symptom scoring.

7.3 RESULTS

There were no significant carryover effects between the first (run-in) and second (washout) placebo values in sequence with any of the measurements [Table 7.1]. Consequently all comparisons were made with reference to the run-in placebo.

Lower Airway Efficacy Markers

There was a significant difference between both treatments and placebo and also a significant difference between active treatments for effects on adenosine monophosphate bronchial challenge - the primary endpoint [Figure 7.1a]. Geometric mean differences (95% CI for difference) were 6.4 fold (2.2 to 18.6) for placebo vs budesonide, 2.9 fold (1.0 to 8.4) for placebo vs montelukast, and 2.1 fold (1.1 to 4.5) for budesonide vs montelukast. For exhaled nitric oxide [Figure 7.1b] there were also significant differences (95% CI for difference) between placebo vs montelukast: 7.9ppb (0.8 to 15.6) and placebo vs budesonide 8.7ppb (0.9 to 16.4). For spirometry data, asthma symptom scores, peak expiratory flow, and rescue agonist usage there were similar numerical trends for improvements with both budesonide and montelukast compared to placebo, although these differences were not statistically significant [Table 7.2].

Upper Airway Efficacy Markers

For nasal nitric oxide [Figure 7.2a] there was a significant difference (95% CI for difference) for placebo vs budesonide 369.3 ppb (15.4 to 723.2) but not with montelukast. Likewise for domiciliary morning nasal peak flow there was significant

difference with placebo vs budesonide 36.8 1/mm (15.9 to 57.5) but not with montelukast [Figure 7.2b]. Both active treatments produced significant effects on eye symptoms and total seasonal allergic rhinitis symptoms (i.e. nose, eye and throat symptoms) compared to placebo whilst only budesonide significantly affected nasal symptoms and daily activity scores [Table 7.2].

Correlations

There were significant correlations between asthma symptoms and morning PEF ($r=-0.25$, $p<0.0001$) and evening PEF ($r=-0.16$, $p<0.001$); and between nasal symptoms and morning nasal PIFR ($r=-0.33$,$p<0.0001$) and evening nasal PIFR ($r=-0.33$,$p<0.0001$).

Table 7.1

Mean (SEM) values for 1st (run-in) and 2nd (washout) placebo in sequence for adenosine monophosphate bronchial challenge PC_{20} (AMP), exhaled nitric oxide (NO), FEV_1, morning peak expiratory flow rate (PEFR), evening peak expiratory flow rate, asthma symptoms, rescue inhaler usage, nasal nitric oxide, morning nasal peak inspiratory flow rate (PIFR), evening peak inspiratory flow rate, total seasonal allergic rhinitis symptoms (SARS), nasal symptoms, eye symptoms, throat symptoms, ocular sodium cromoglycate use (Ocular cromoglycate), and effects of symptoms on patients' daily activity (Daily Activity). Values for AMP PC_{20} are given as geometric mean.

	First PL	Second PL		First PL	Second PL
AMP (mg/ml)	23.5 (6.4)	34.5 (8.4)	Nasal Nitric Oxide (ppb)	949.8 (97.5)	797.5 (87.8)
Exhaled Nitric Oxide (ppb)	17.4 (2.0)	13.6 (1.8)	Morning PIFR (l/min)	98.8 (5.3)	110.4 (4.7)
FEV1 (% predicted)	89.3 (2.6)	90.3 (2.2)	Evening PIFR (l/min)	114.4 (6.6)	122.1 (5.8)
Morning PEFR (l/min)	399.2 (7.7)	398.4 (6.4)	SARS (units)	6.7 (0.9)	4.3 (0.7)
Evening PEFR (l/min)	393.6 (20.4)	425.4 (17.8)	Nasal Symptoms (units)	4.5 (0.6)	2.9 (0.5)
Asthma symptoms (units)	1.2 (0.2)	0.8 (0.2)	Eye Symptoms (units)	2.4 (0.4)	1.4 (0.4)
Rescue inhaler (Puffs/day)	2.0 (0.5)	1.9 (0.4)	Throat Symptoms (units)	0.6 (0.2)	0.7 (0.1)
Daily Activity (units)	2.3 (0.5)	3.0 (0.4)	Ocular cromoglycate (units)	0.7 (0.2)	0.3 (0.1)

Table 7.2
Mean (SEM) values after run-in placebo (PL) and after active treatment with budesonide (BUD) or montelukast (MON). Data are for FEV_1, morning peak expiratory flow rate (PEFR), evening PEFR, asthma symptoms, rescue inhaler usage, effects of symptoms on patients' daily activity (Daily Activity), evening peak inspiratory flow rate (PIFR), total seasonal allergic rhinitis symptoms (SARS), nasal symptoms, eye symptoms, throat symptoms, and ocular sodium cromoglycate use (Ocular cromoglycate). Asterisk denotes a significant ($p<0.05$) difference between active treatments and placebo.

Treatment	PL	BUD	MON	Treatment	PL	BUD	MON
FEV1 (% predicted)	89.3 (2.6)	94.3 (2.1)	91.3 (2.1)	Evening PIFR (l/min)	114.4 (6.6)	135.7 (5.6)	124.0 (5.5)
Morning PEFR (l/min)	399.2 (7.7)	424.3 (6.2)	401.1 (6.1)	SARS (units)	6.7 (0.9)	1.8 * (0.7)	3.5 * (0.7)
Evening PEFR (l/min)	393.6 (20.4)	441.7 (17.3)	432.5 (16.8)	Nasal Symptoms (units)	4.5 (0.6)	1.3 * (0.5)	2.8 (0.5)
Asthma Symptoms (units)	1.2 (0.2)	0.5 (0.2)	0.9 (0.2)	Eye Symptoms (units)	2.4 (0.4)	0.4 * (0.3)	0.9 * (0.3)
Rescue inhaler (Puffs/day)	2.0 (0.5)	0.7 (0.4)	1.5 (0.4)	Throat Symptoms (units)	0.6 (0.2)	0.4 (0.1)	0.6 (0.1)
Daily Activity (units)	2.3 (0.5)	1.2 * (0.4)	1.5 (0.4)	Ocular Cromoglycate (units)	0.7 (0.2)	0.2 (0.1)	0.3 (0.1)

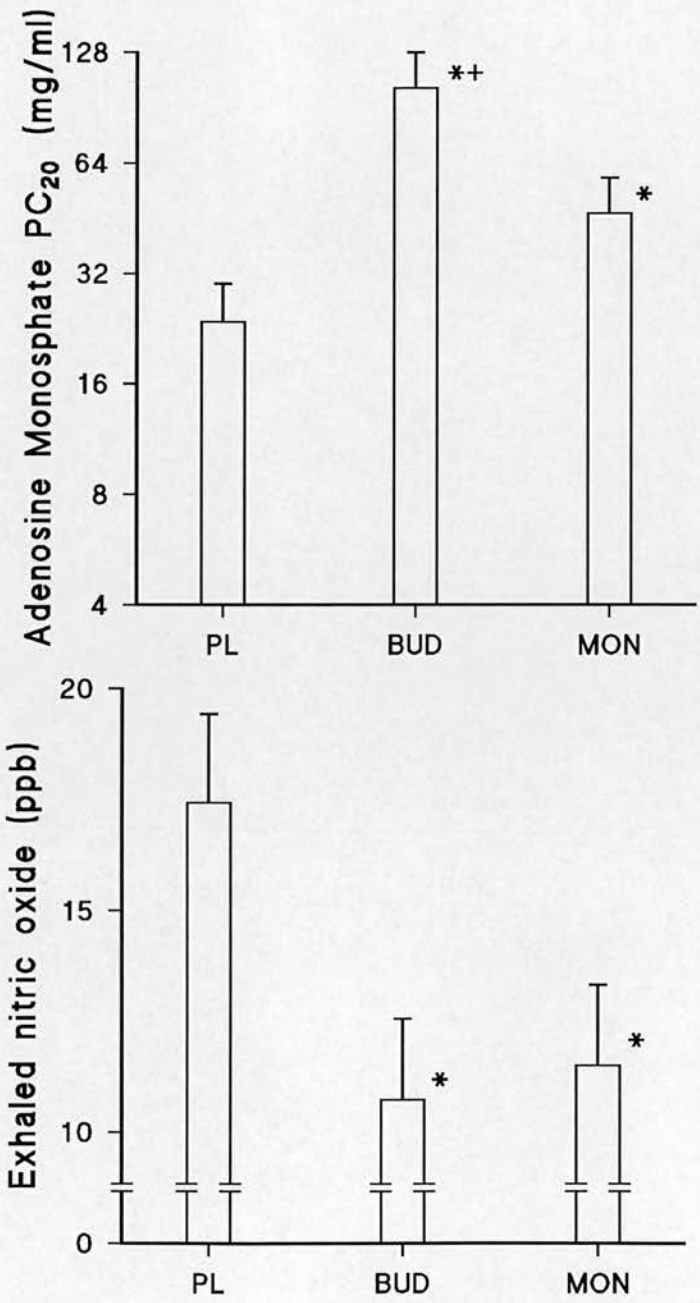


Figure 7.1
(a) Geometric mean (SE) for adenosine monophosphate bronchial challenge PC_{20} for placebo (PL), inhaled and intra-nasal budesonide (BUD) and oral montelukast (MON). (b) Means (SE) for exhaled nitric oxide (ppb). Asterisk denotes significant ($p<0.05$) difference between active treatment and placebo. Cross denotes significant difference between active treatments.

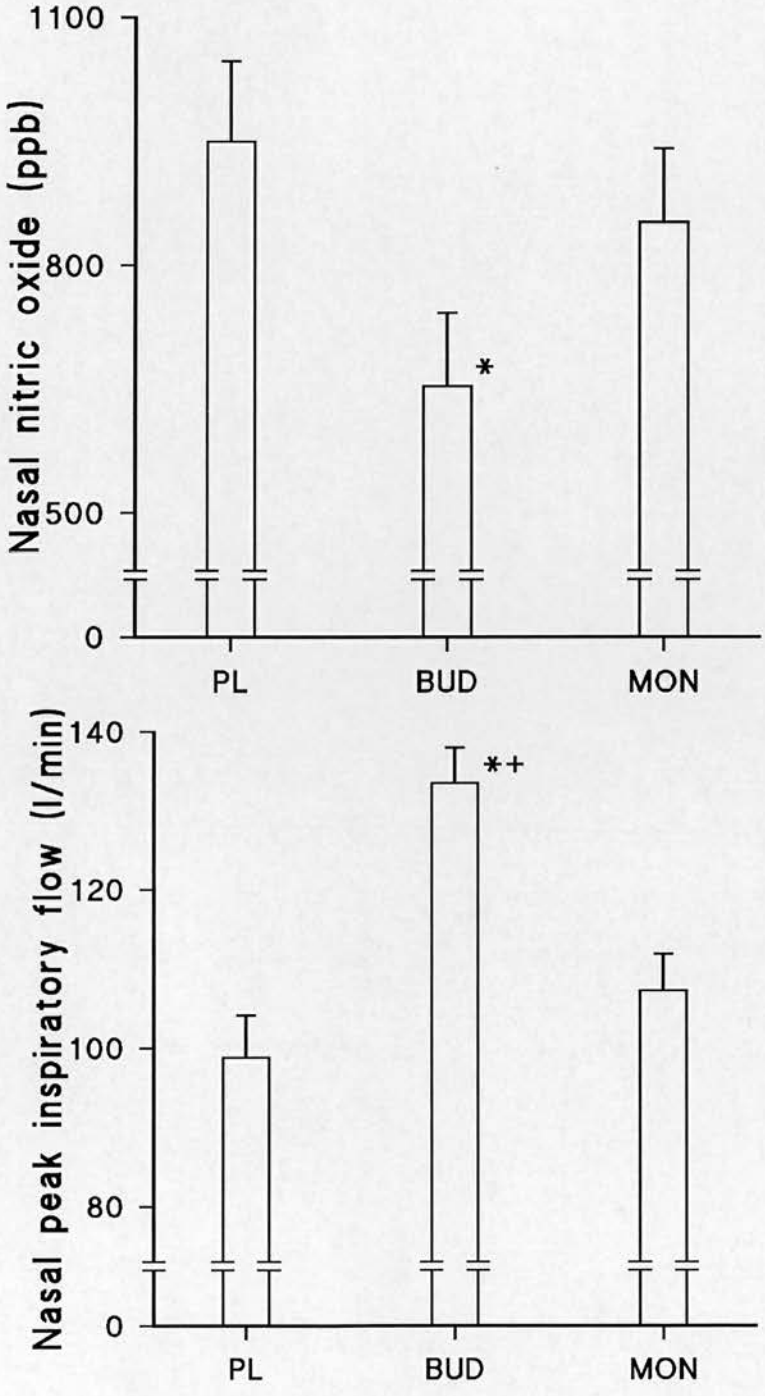


Figure 7.2
Means (SE) for (a) morning nasal peak inspiratory flow rate (l/min) and (b) nasal nitric oxide (ppb) for placebo (PL), inhaled and intra-nasal budesonide (BUD) and oral montelukast (MON). Asterisk denotes significant ($p<0.05$) difference between active treatment and placebo.

7.4 DISCUSSION

This study has shown that in patients with concomitant seasonal allergic rhinitis and asthma, the use of oral montelukast or inhaled plus intra-nasal budesonide both exhibited beneficial effects on markers of lower airway inflammation (adenosine monophosphate bronchial challenge and exhaled nitric oxide). However, only inhaled plus intra-nasal budesonide therapy showed a significant improvement on upper airway parameters (nasal nitric oxide and nasal peak inspiratory flow rate). Both active treatments exhibited significant improvements in total seasonal allergic rhinitis symptoms and on eye symptoms alone, whilst budesonide improved nasal symptoms and daily activity scores.

The results with exhaled nitric oxide are in keeping with those of Kharitinov et al[369], who showed that altering the dose of budesonide by 200μg per day resulted in detectable changes in exhaled nitric oxide production. They showed budesonide 800μg per day given via a Turbuhaler caused significant suppression of nitric oxide compared with placebo[369]. There are also data to suggest that leukotriene receptor antagonists reduce exhaled nitric oxide when given in addition to inhaled corticosteroids[106]. More recently Bisgaard et al[107] showed that montelukast reduced exhaled levels in asthmatic children by 20%, also after 2 weeks of therapy, and this effect was independent of concurrent steroid treatment. Regarding nasal nitric oxide production, studies have demonstrated suppression with intranasal corticosteroids[97].

There are conflicting data on the effects of leukotriene receptor antagonist, given as

monotherapy, on bronchial hyperreactivity. In a dose-response study for 12 weeks, montelukast exhibited a 1.3 fold protection against methacholine challenge although this was not significant compared to placebo[292]. In a randomised, double blind, crossover study, zafirlukast 20 mg bid and fluticasone propionate 100μg bid for 2 weeks exhibited 1.7 fold and 2.8 fold protection respectively against histamine challenge[370]. In a subgroup analysis of a randomised crossover trial, there was 2.4-fold protection against methacholine hyperreactivity after 2 weeks of zafirlukast 20mg bid compared to placebo[371]. Studies with pranlukast have also shown protection against methacholine and allergen challenge, and, in one study, a reduction in bronchial mucosal inflammatory cells[284,372,373]. As leukotrienes are inflammatory mediators, it is not surprising that montelukast exhibited bronchoprotection with adenosine monophosphate although the effect was less than that of budesonide (a 6.4 and 2.9 fold difference from placebo respectively).

Montelukast was shown to have significant beneficial effects on allergic rhinitis symptom scores in this study, which is in keeping with Malmstrom et al[312], Donnelly et al[311] and Grossman et al[310]. The superiority of an intra-nasal corticosteroid to a leukotriene receptor antagonist has previously been shown in a study comparing 20mg twice daily oral zafirlukast to 200μg intra-nasal beclomethasone dipropionate[374]. Nasal biopsies taken after 6 weeks of treatment showed decrease in eosinophilia with beclomethasone dipropionate, compared to pre-season biopsies, but not with zafirlukast. However, in that study, there was no significant difference with zafirlukast and placebo

in terms of patients symptom scores[374].

Mild asthmatic patients were investigated as leukotriene antagonists and once daily budesonide are only recommended as monotherapy in such patients. As a result the patients in this study had near normal spirometry with a mean FEV_1 of 91% predicted and therefore it was not surprising to see little improvement in lung function. Likewise with asthma symptom scoring there was no significant improvement with either treatment although there was a numerical trend with topical budesonide having better symptom control and less β_2 agonist use than montelukast or placebo. This study was powered on bronchial challenge testing, but had more severe patients been investigated or used greater numbers of patients these differences may have been significant.

Although bronchial challenge testing and nasal peak inspiratory flow were the only parameters to show significantly greater efficacy with the corticosteroid than the leukotriene antagonist, there was a similar numerical trend for other upper and lower airway markers. It is conceivable, therefore, that such differences would have become significant with a larger sample size. The primary end point was adenosine monophosphate bronchial challenge and the study was therefore powered on this measure. The sample size had been calculated to be adequate to detect a one doubling dose difference, as this was deemed clinically relevant. Indeed a significant difference was detected between placebo and active treatments and between both active treatments.

It would still have been more satisfactory to include a larger number of patients into the study. However, this study was performed during the pollen season which is particularly short in Dundee and therefore there was only a limited time to recruit patients. Also the entry criteria were fairly tight. Patients had to be suffering from both allergic rhinitis and asthma and they had to have a provocation dose of adenosine monophosphate causing a 20% fall in FEV_1 of less than 200mg/ml. They also had to have relatively mild disease as indicated by a pre-recruitment inhaled corticosteroid dose of less than or equal to 400μg per day.

Previous studies have also shown fluticasone propionate to have better control of symptoms and lung function than zafirlukast as monotherapy[375]. This is probably due to the greater anti-inflammatory potency of corticosteroids, which have a broad spectrum of activity on the inflammatory cascade, rather than on one of the inflammatory mediators[99].

There was also a significant correlation between nasal peak inspiratory flow rates and nasal symptoms in this study. Although previous studies have shown comparative results between nasal peak inspiratory flow rate, and rhinomanometry or acoustic rhinometry[367], this is one of the first studies to examine its role in the domiciliary setting.

CHAPTER 8

A COMPARISON OF INHALED FORMOTEROL AND BUDESONIDE ALONE OR IN COMBINATION ON INFLAMMATORY MARKERS IN ASTHMATIC ADULTS

8.1 INTRODUCTION

Having shown that leukotriene receptor antagonists exhibit beneficial effects in terms of disease control, surrogate measures of inflammation and patients symptoms, it was important to also consider the properties of long-acting β_2 agonists. As discussed previously (see section 1.5.1), long-acting β_2-agonists such as salmeterol and formoterol are used on a regular basis as second-line controller therapy in addition to inhaled corticosteroids in order to improve symptomatic control of asthma and exacerbation rates. However, in view of the concerns that the use of regular long-acting β_2-agonists may potentially mask an increase in underlying inflammatory processes, it was important to assess the anti-inflammatory properties of long-acting β_2 agonists and inhaled corticosteroids.

Pauwels et al[62] reported the results of the FACET study which evaluated the effects of adding inhaled formoterol to both lower and higher doses of the inhaled budesonide. They found beneficial effects with both high dose budesonide alone, and with the addition of inhaled formoterol to low or high dose budesonide. Furthermore, the addition of formoterol improved symptoms and lung function and did not increase the number of exacerbation rates. In order to mirror that study[62], the effects of low and high doses of formoterol and budesonide were evaluated when given alone or in combination. Measurements of lung function and surrogate inflammatory markers (adenosine monophosphate bronchial challenge, exhaled nitric oxide and serum eosinophilic cationic protein) were made as well as patients symptoms scores[112]. However, it was

felt important to consider, at the same time, whether the effects on inflammatory markers or lung function mirrored the patients' preference for monotherapy or combination therapy. Patients with stable mild to moderate asthma who were already using regular inhaled corticosteroids were recruited in line with the accepted asthma management guidelines.

As stated above budesonide and formoterol were chosen in order to reflect the FACET study. They are both made by the same company and are dispensed by the same delivery device, the Turbuhaler, which is a dry powder reservoir inhaler. This was more convenient for the patients to be studied and reduced the influence of lung delivery on the effectiveness of treatment.

8.2 METHODS

Patients

Fifteen stable atopic asthmatic patients (7 female) mean (±SE) age 32.4 (3) years, all taking inhaled corticosteroids mean dose 473 (57)μg per day were randomised. The subjects had mild to moderate asthma and were using inhaled corticosteroids in a dose of less than 1000μg per day. Baseline spirometry showed mean FEV_1 2.74 (0.20) L, 75.2 (2.8) percent predicted and FEF_{25-75} 2.29 (0.23) l/s, 52.4 (4.0) percent predicted at recruitment. All patients were required to be atopic on Phadiotop testing, and to be responsive to adenosine monophosphate (AMP) with a geometric mean provocation concentration producing a 20% fall in FEV_1 (PC_{20}) of less than 200mg/ml. One subject was using oral theophylline therapy and one was taking long-acting β_2-agonist therapy with formoterol.

Protocol

The study had a placebo controlled, double-blind, double-dummy, cross-over design. The subjects attended the laboratory for the initial screening visit in the morning between 9am and 11am and all the subsequent visits were performed within the same 2 hour window. The patients withheld their long-acting β_2-agonist therapy for 48 hours and oral theophylline therapy for 72 hours and short-acting β_2-agonist therapy for at least 12 hours prior to this screening visit. An adenosine monophosphate (AMP) bronchial challenge test was performed. The patients who had a provocative concentration of AMP producing a 20% fall in FEV_1 (PC_{20}) of less than 200mg/ml,

entered the run-in phase. From the start of the run-in until the end of the trial all therapy with theophylline, long-acting β_2-agonists and inhaled corticosteroids were stopped. Inhaled ipratropium bromide 2 puffs (Atrovent Forte, 40μg per puff, Boehringer Ingelheim, Bracknell UK) was used as required for symptomatic relief purposes as 1st line rescue, with inhaled salbutamol as 2nd line rescue. During subsequent visits the subjects withheld ipratropium bromide or salbutamol for at least 12 hours as well.

At the start of the initial 1 week placebo run-in the patients were given a placebo budesonide Turbohaler® (Astra Pharmaceuticals, Kings Langley UK) along with a placebo formoterol Turbohaler® to use both on a regular once daily basis at 8pm. The patients were taught in the correct technique for using the Turbohaler® by making sure that they were able to generate an inspiratory flow of at least 60 L/min using a Turbohaler® training device (Astra Draco, Lund Sweden).

The subjects attended the laboratory for the next visit after the 7-day placebo run-in period having taken the last dose of the placebo Turbuhalers at 8pm the previous evening. The patients collected their overnight urine for a 10 hour cortisol collection from 10pm till 8am prior to attending the department. At this first study visit the patients had 5ml of blood taken for the measurement of ECP followed by measurement of exhaled nitric oxide. After that the subjects performed baseline spirometry followed by AMP challenge. The subjects were then given their randomised treatments with either a) inhaled formoterol 12μg 1 puff once daily (as Oxis Turbohaler, eformoterol

fumarate 12 μg per puff, Astra Pharmaceuticals, Kings Langley UK) along with a placebo Turbohaler® 1 puff or b) inhaled budesonide 400μg 1 puff once daily (as Pulmicort Turbohaler, budesonide 400μg per puff, Astra Pharmaceuticals, Kings Langley UK) along with a placebo Turbohaler® 1 puff or c) inhaled formoterol 12μg 1 puff with inhaled budesonide 400μg 1 puff both once daily. Both inhalers were taken as 1 puff at 8pm each night for the first 2 weeks. The subjects were also instructed to record the best of three values for their morning and evening peak flows the latter prior to taking the evening medication in a diary card using a Mini-Wright peak expiratory flow meter (Clement Clarke Ltd., Harlow UK). The subjects also recorded the total number of puffs of ipratropium bromide or salbutamol rescue therapy taken for each day.

The subjects attended the laboratory for the next visit after two weeks of each randomised treatment, 12 hours after taking the 14th dose of their randomised treatment, bringing along their overnight urinary cortisol collection and diary cards. The same process was repeated for the measurement of NO, spirometry and AMP challenge except that a sample for ECP was not collected at this occasion. The patients were then instructed to start taking 2 puffs from the same randomised inhalers each evening for a further 2 weeks and to record their peak flow and rescue inhaler use in a new diary card. The subjects then returned to the laboratory after further two weeks, 12 hours after taking their 28th dose (14th dose of the 2 puff per day period). At this third visit a sample for ECP was collected along with measurement of exhaled NO, spirometry and AMP

challenge.

The subjects then entered a 7 day washout period where they again used the two placebo Turbuhalers at night. The same process was repeated with the next randomised treatment for 2 weeks (1 puff) and 4 weeks (2 puffs). This was again followed by a further 7-day placebo washout period and a subsequent randomised 4 week treatment block. Patient preference was evaluated at the second and third sequences after 2 and 4 weeks of treatment.

Measurements

Spirometry

Adenosine Monophosphate Bronchial Challenge

Measurement of Eosinophilic Cationic Protein

Measurement of Exhaled Nitric Oxide

Measurement of Urinary Free Cortisol

Diary Card Data

Patient preference

Statistical Analysis

The study was powered at 80% to detect a 1 doubling dose (2-fold) difference in AMP PC_{20}. The data for PC_{20}, exhaled nitric oxide and eosinophilic cationic protein were all log transformed to normalize their distribution, prior to analysis. The parameters were analysed as geometric mean values and as fold differences (with 95% confidence

intervals). The first visits for all three randomised treatments (i.e. after placebo run-in or placebo washout periods) were compared according to their order in sequence. In addition the first visits for all 3 treatments were compared irrespective of sequence - i.e. prior to formoterol alone, budesonide alone and formoterol + budesonide respectively. A pooled placebo was then calculated and used for comparison with active treatments.

The statistical analysis was performed for within treatment effects (i.e. comparing each active treatment at 2 and 4 weeks to pooled placebo) and between treatment effects (i.e. comparing the 3 active treatments at 2 weeks and 4 weeks). The statistical analysis was performed by multifactorial analysis of variance (MANOVA) using subject, treatment, visit and sequence as factors. This was followed by Bonferroni multiple-range testing (set at 95% CI) to obviate multiple pair-wise comparisons, in order not to confound the overall alpha error ($p<0.05$, two-tailed). Hence all parameters are described as being significant ($p<0.05$) or not according to the Bonferroni test. The patient preference was analysed +using Friedman's Rank test.

8.3 RESULTS

All subjects completed the study. Two subjects complained of hoarseness of voice, both during the steroid limbs. They were reminded of mouth washing after inhalation and they did not complain of the symptoms during the subsequent limbs. The urinary free cortisol did not show any significant difference pooled placebo and active treatments (as mean (SE)); PL: 24.6 nmol (2.6), FM12: 20.1 nmol (3.3), FM24: 22.6 (5.4), BUD400: 29.4 nmol (6.2), BUD800: 22.6 nmol (5.1), FM12+BUD400: 27.5 nmol (5.7), FM24+BUD800: 18.2 nmol (2.5).

Domiciliary Diary Cards

The domiciliary peak flow recordings [Figure 8.1] showed significant improvements with BUD + FM together as compared to FM or BUD alone. For morning peak flow (as mean over 4 weeks); FM+BUD 473 l/min vs. FM 460 l/min (95% CI for difference 2 to 22), vs. BUD 453 (95% CI for difference 9 to 29). Similarly for evening peak flow; FM+BUD 486 vs. FM 472 (95% CI for difference 5 to 22), vs. BUD 476 (95% CI for difference 1 to 19). Rescue use of salbutamol was much less than for ipratropium bromide. The rescue inhaler usage of ipratropium bromide was significantly lower after FM+BUD as compared to FM but not compared to BUD, whilst for salbutamol usage was significantly lower after FM+BUD as compared to BUD but not compared to FM.

Baseline spirometry

The pre-challenge spirometry [Figure 8.2] showed significant improvements in FEV_1

and FEF_{25-75} with all active treatments as compared to baseline but there were no significant differences between treatments [Table 8.1].

Placebo visits

All parameters did not show any significant differences when comparing the visits after placebo according to sequence, or irrespective of sequence according to treatments (i.e. placebo prior to FM, prior to BUD, or prior to FM+BUD) [Table 8.2]

Adenosine monophosphate bronchial challenge

The adenosine monophosphate bronchial challenge test [Figure 8.3] showed that all active treatments afforded significantly better protection as compared to placebo [Table 8.3]. There was a dose-response effect in terms of a significant difference between low and high doses of FM+BUD for visit 2 vs. visit 3: i.e. FM12+BUD400 vs. FM24+BUD800 - a 2.4-fold difference (95% CI 1.0 to 5.5-fold).

There were no significant differences between the three low-dose treatments at visit 2 after 2 weeks. For high-dose treatment after 4 weeks there was significantly better bronchoprotection with both BUD containing regimens compared to FM alone: FM24 vs. BUD800 - a 2.5-fold difference (95% CI 1.1 to 5.4-fold), vs. FM24+BUD800 - a 3.2-fold difference (95% CI 1.5 to 7-fold). There was no significant difference between BUD alone and BUD plus FM at either 2 or 4 weeks. Individual rank order for effects on AMP PC_{20} showed significant ($p=0.004$) differences with both BUD containing limbs compared to FM alone.

Exhaled nitric oxide [Figure 8.3]

For FM alone there were no significant differences at either dose compared to placebo. Both doses of BUD alone or combination therapy exhibited significantly lower NO levels compared to placebo [Table 8.3]. For BUD alone at both doses and for combination therapy at high dose there were significant reductions in exhaled NO compared to FM alone: FM12 vs. BUD400 a 1.7-fold difference (95% CI 1.2 to 2.4-fold), FM24 vs. BUD800 a 2.1-fold difference (95% CI 1.1 to 3.4-fold), and FM24 vs. FM24+BUD800 a 1.8-fold difference (95% CI 1.1 to 2.9-fold). Individual rank order for effects on exhaled nitric oxide showed significant ($p=0.004$) differences with both BUD containing limbs compared to FM alone.

Eosinophilic cationic protein

There was no significant reduction in ECP (measured only at visit 3) after FM treatment compared to placebo (95% CI for fold difference included unity), while there were significant reductions in ECP after both BUD containing limbs [Table 8.3].

Patient preference

The patients preferred the combined treatment as their first choice followed by FM alone and then BUD alone in rank order: FM + BUD > FM > BUD $p<0.0005$ [Figure 8.4].

Table 8.1

Spirometry prior to adenosine monophosphate challenge comparing the active treatments to the mean placebo. The values are arithmetic mean (SE). Asterisk denotes significant ($p<0.05$) difference between active treatment and placebo.

	FEV_1 (l)	**FEV_1 (% predicted)**	**FEF_{25-75} (l/s)**	**FEF_{25-75} (% predicted)**
Placebo	2.7 (0.25)	73.3 (4.4)	2.3 (0.3)	53.3 (7.7)
FM (12µg)	3.0 * (0.2)	82.2 * (4.4)	2.8 * (0.2)	65.3 * (4.4)
FM (24µg)	3.1 * (0.3)	83.6 * (4.9)`	2.9 * (0.3)	68.4 * (6.1)
BUD (400µg)	3.0 * (0.2)	82.2 * (4.4)	2.8 * (0.3)	64.5 * (5.2)
BUD (800µg)	2.9 * (0.2)	80.7 * (4.4)	2.7 * (0.2)	63.3 * (5.3)
FM+BUD (12µg+400µg)	3.0 * (0.2)	81.9 * (4.1)	2.8 * (0.3)	65.0 * (5.6)
FM+BUD (24µg+800µg)	3.0 * (0.2)	83.2 * (3.4)	2.9 * (0.3)	66.8 * (5.5)

Table 8.2

Measurements at visit 1 after placebo prior to each of the randomised treatments. The values for spirometry are arithmetic means (SE) and the values for PC_{20}, NO and ECP are geometric means (SE). There were no significant differences in placebo values for each treatment

	FM	**BUD**	**FM+BUD**
FEV_1 **(l)**	2.69 (0.23)	2.67 (0.27)	2.70 (0.25)
FEV_1 **(% predicted)**	73.8 (4.9)	72.3 (4.6)	73.8 (4.6)
FEF_{25-75} **(l/s)**	2.37 (0.24)	2.30 (0.28)	2.32 (0.28)
FEF_{25-75} **(% predicted)**	54.8 (4.9)	52.6 (5.1)	52.8 (5.2)
AMP PC_{20} **(mg/ml)**	22.1 (7.5)	15.9 (8.0)	13.8 (9.3)
Nitric oxide **(ppb)**	14.7 (2.9)	13.1 (2.2)	18.1 (3.8)
ECP **(µg/l)**	12.3 (1.8)	13.5 (2.2)	14.6 (3.2)

Table 8.3

Comparison of inflammatory markers between active treatments and mean placebo All values are geometric means with fold differences from placebo (95% confidence intervals for fold-difference which include unity are non-significant). Asterisk denotes significant difference ($p<0.05$) between active treatments and pooled placebo

	PC_{20} AMP (mg/ml)	**Exhaled Nitric Oxide (PPB)**	ECP **(µg/l)**
Placebo	20	15.9	14.1
FM 12µg	66 * 3.3-fold difference (95% CI 1.4 to 8.2)	13.9 1.1-fold difference (95% CI 0.8 to 1.5)	(Not Measured)
FM 24µg	82 * 4.1-fold difference (95% CI 1.7 to 10.2)	15.5 1-fold difference (95% CI 0.8 to 1.3)	9.6 1.5-fold difference (95% CI 0.8 to 2.6)
BUD 400µg	109 * 5.5-fold difference (95% CI 2.5 to 12.3)	8.3 * 1.9-fold difference (95% CI 1.3 to 2.8)	(Not Measured)
BUD 800µg	201 * 10.2-fold difference (95% CI 4.6 to 22.5)	7.4 * 2.1-fold difference (95% CI 1.5 to 3.1)	9.7 * 1.5-fold difference (95% CI 1.0 to 2.1)
FM+BUD 12µg+400µg	111 * 5.6-fold difference (95% CI 2.4 to 13)	11.5 * 1.4-fold difference (95% 1.1 to 1.8)	(Not Measured)
FM+BUD 24µg+800µg	261 * 13.2-fold difference (95% CI 5.7 to 30.6)	8.8 * 1.8-fold difference (95% CI 1.4 to 2.4)	9.9 * 1.4-fold difference (95% CI 1.1 to 1.9)

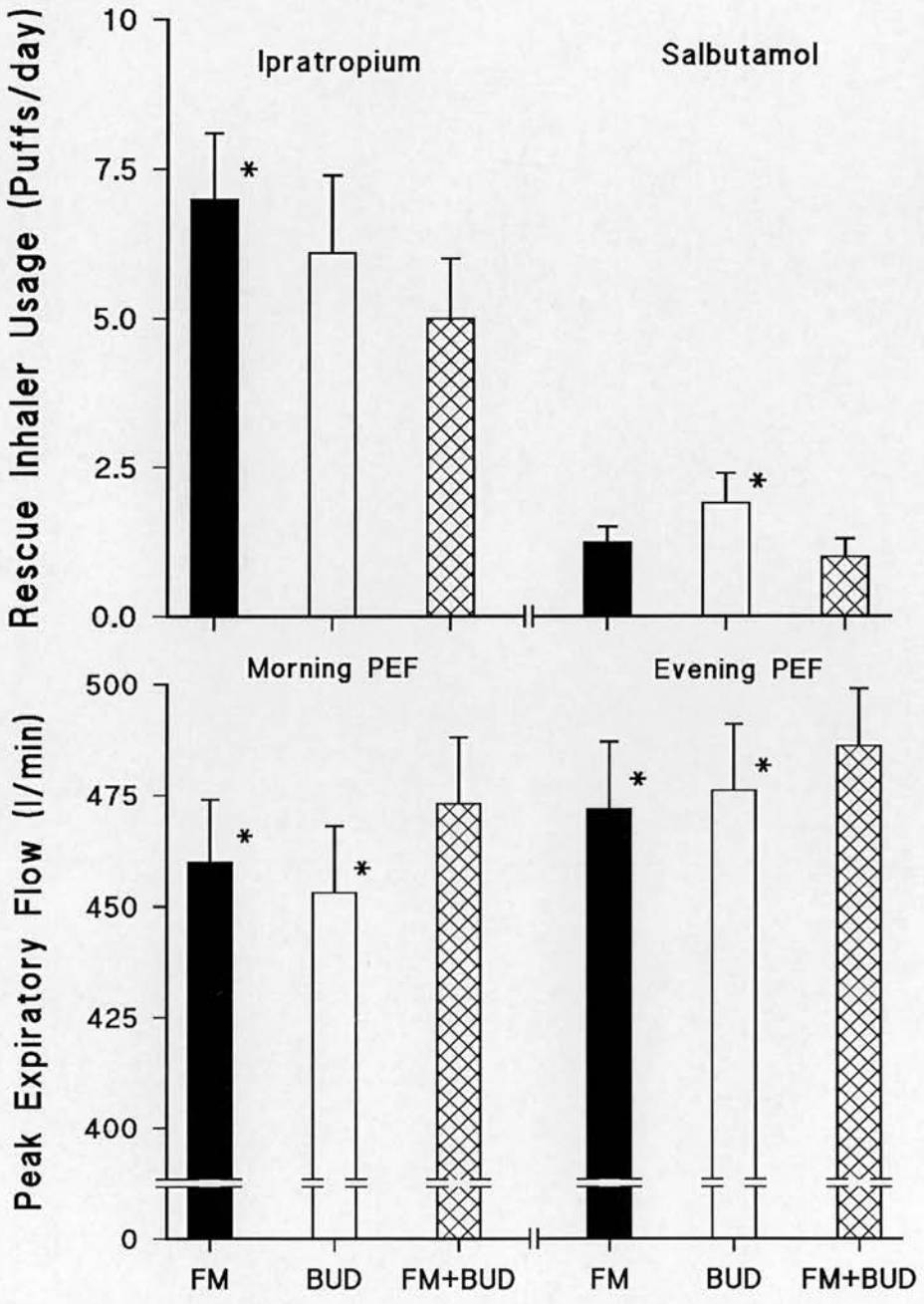


Figure 8.1
Overall means (SE) for the 4 week treatment period for a) rescue inhaler usage of ipratropium bromide (1st line) and salbutamol (2nd line) and b) domiciliary peak expiratory flow rate for morning and evening recordings for formoterol (FM), budesonide (BUD), and combination (FM+BUD). Asterisk denotes as significant ($p<0.05$) difference from combination therapy.

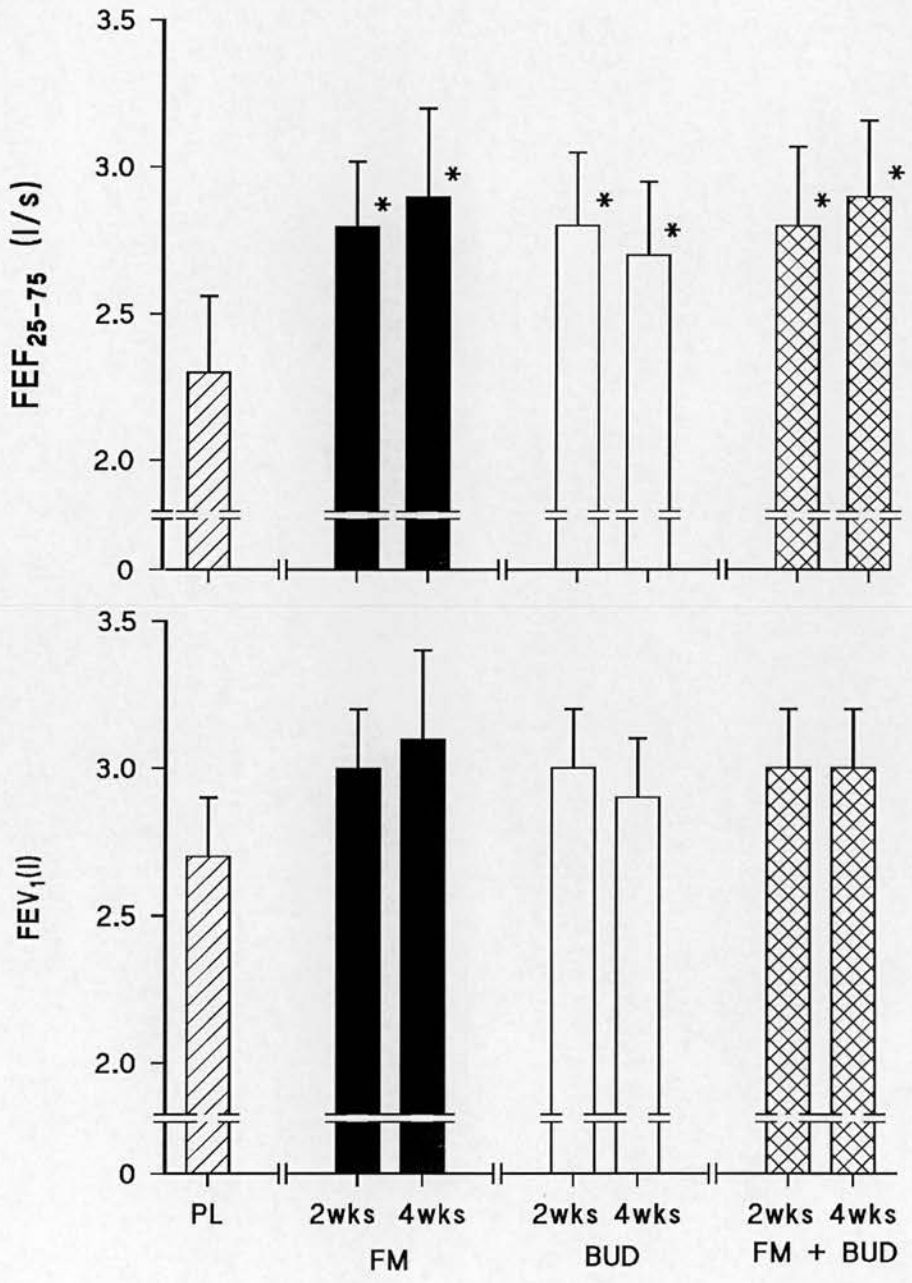


Figure 8.2
Pre-challenge spirometry as (a) FEF_{25-75} and (b) FEV_1 for the active treatments compared to pooled placebo. The values are arithmetic means (± SE)

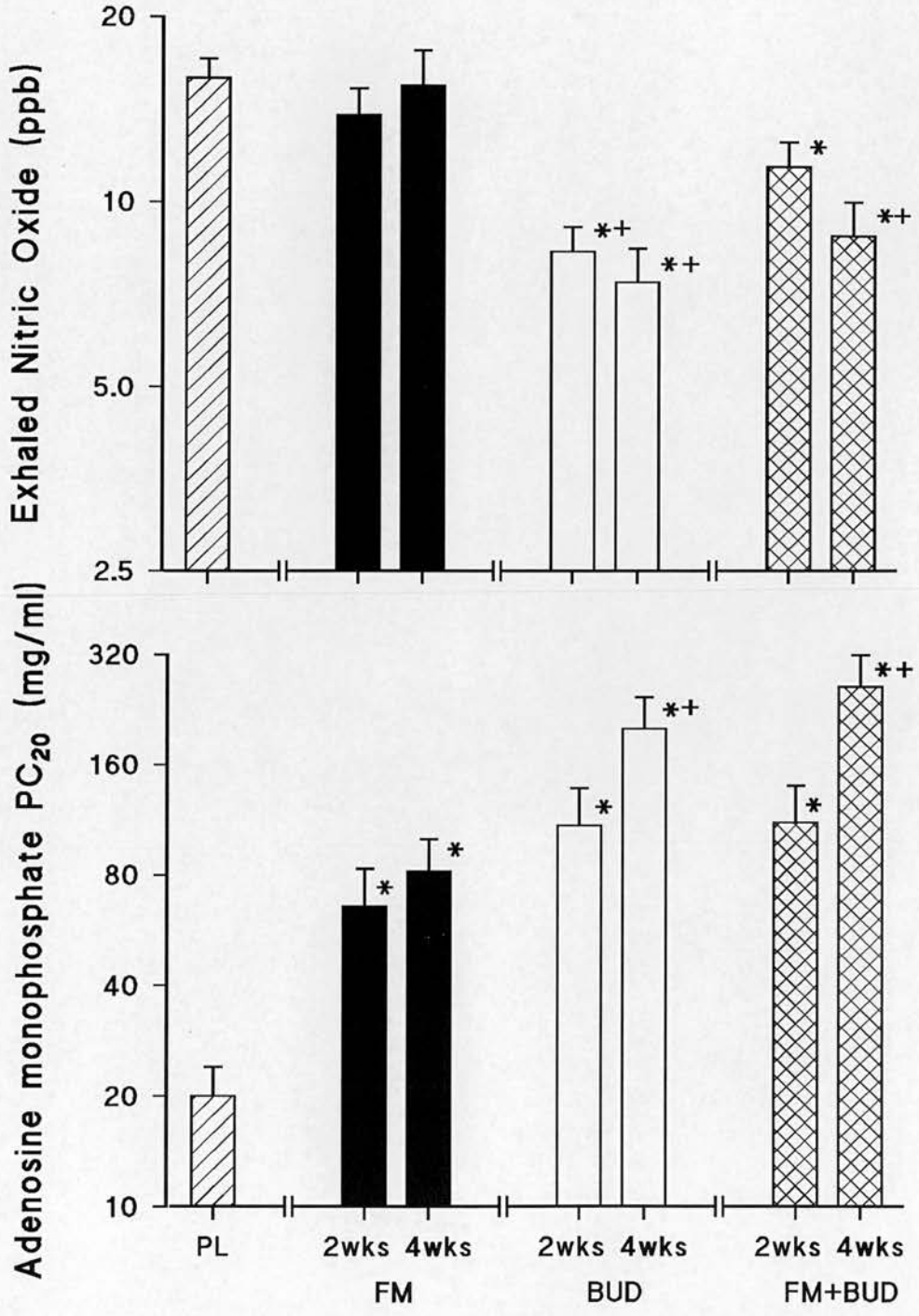


Figure 8.3
(a) Adenosine monophosphate bronchial challenge for active treatments compared to pooled placebo. (b) Exhaled nitric oxide for active treatments compared to pooled placebo. The values are geometric means (± SE).

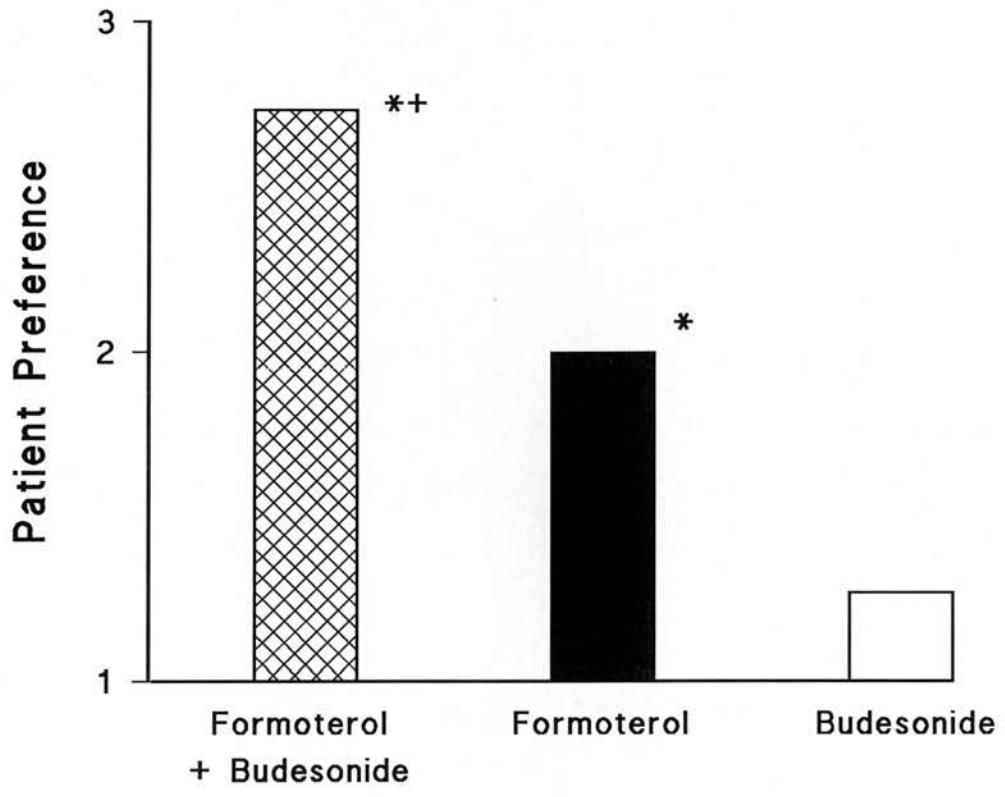


Figure 8.4
The rank order for patient preference between the three treatments. The results are shown according to the marking system: first choice '3' marks, second choice '2' marks and third '1' mark.

8.4 DISCUSSION

The main findings from this study were: (a) Patients preferred combined treatment with formoterol and budesonide to budesonide alone, which was mirrored by effects on domiciliary peak expiratory flow and rescue medication. (b) Despite patient preference and improved peak flow with combined therapy, this was not associated with improved anti-inflammatory control (on exhaled nitric oxide, serum eosinophilic cationic protein and adenosine monophosphate challenge) as compared to budesonide monotherapy. (c) Formoterol monotherapy had no significant anti-inflammatory effects on exhaled nitric oxide and exhibited antagonism against adenosine monophosphate challenge to a lesser degree than budesonide monotherapy. (d) Budesonide exhibited a dose-related effect on adenosine monophosphate challenge when given as monotherapy or combined therapy in keeping with its anti-inflammatory activity. (e) All 3 treatments exhibited equivalent improvements in FEV_1 and FEF_{25-75} .

In the study of Pauwels et al[62] adding formoterol to budesonide produced improvements in peak expiratory flow and reductions in exacerbation rates. The data from the study in this thesis showed that formoterol conferred additive effects on peak expiratory flow, but exhibited no significant anti-inflammatory activity on its own. Similarly the addition of salmeterol to beclomethasone has been shown to have no significant effects on bronchoalveolar lavage cell profile[376]. However, recent data have suggested that monotherapy with formoterol may reduce the number of mast cells and eosinophils in bronchial biopsies from patients with mild atopic asthma[270].

Pauwels et al[62] also showed a proportionally greater reduction in exacerbation rates by increasing the dose of budesonide as monotherapy(from 200 to 800μg per day) as compared to the additive effects of formoterol. This is in keeping with the data reported here as doubling the dose of budesonide exhibited further protection against adenosine monophosphate challenge irrespective of the addition of formoterol. These results, showing greater suppression of exhaled nitric oxide and bronchial hyperreactivity with budesonide alone compared to formoterol alone, are similar to observations of Verberne et al[377] and Simons et al[378]. These authors showed beclomethasone dipropionate to be superior to salmeterol for effects on bronchial hyperreactivity (as methacholine challenge) and exacerbation rates. This emphasizes the importance of optimizing anti-inflammatory control with inhaled corticosteroids before considering adding in regular long-acting β_2-agonist treatment.

The reason for the synergistic effects of inhaled corticosteroids and long acting β_2 agonists on airways inflammation may be explained by their differential effects on inflammatory cells. Glucocorticoids have been shown to have no effects on mast cell mediator release although they are effective at inhibiting basophil mediator release. Whereas the opposite is found for β2-agonists, which only attenuate the release of mast cell mediators[379-381]. Thus the combination will block both types of inflammatory cells. Furthermore, Knightingale et al[382] suggested that long-acting β_2 agonists may have a mast cell stabalising effect as formoterol had a greater bronchoprotective effect than salbutamol in terms of adenosine monophosphate bronchial challenge, but both drugs

were equivalent in terms of histamine challenge. However in contrast to this, Taylor et al[383] found albuterol to have a greater effect on adenosine monophosphate than histamine challenge but there was no significant difference between these tests with salmeterol.

The results of this study show a patient preference for the combination treatment compared to corticosteroid alone, which was associated with increased peak expiratory flow rates and lower rescue requirements. This preference may reflect the patients' perception of a rapid onset of bronchodilator response with formoterol as compared to the more gradual onset of action with corticosteroid. Combining long-acting β_2-agonists and inhaled corticosteroids in the same inhaler formulation (e.g. *Flixotide* plus *Serovent* as *Seretide*, Glaxo-Wellcome, Uxbridge, UK) might conceivably result in improved compliance, at least when initiating therapy.

Another finding of the study was that none of the corticosteroid containing regimens caused a significant fall in urinary free cortisol. Thus, increasing the dose of inhaled budesonide up to 800μg per day was not associated with significant systemic bioactivity. This finding is in keeping with data from a study which demonstrated no significant effects of 400μg or 800μg of budesonide in terms of urine and serum cortisol excretion when given once daily[334].

There are several limitations of this study for example a small patient sample was

investigated who had mild to moderate asthma. Mild to moderate patients were chosen as the intention was to evaluate the comparative effects of budesonide and formoterol, and consequently with the washout there was a potential 6 week period when the patients might conceivably not be taking any inhaled corticosteroids. In order to make sure that the patients did not develop acute worsening of their disease control, it was decided to evaluate those who were using less than 1000μg per day of inhaled corticosteroids. Budesonide was used via a Turbohaler as it is licensed in the UK for use as once daily therapy in a dose up to 800μg per day. Formoterol was used at night once daily as it is often taken in this way for control of nocturnal dips.

CHAPTER 9

A COMPARISON OF SALMETEROL AND MONTELUKAST AS SECOND-LINE THERAPY IN ASTHMATIC PATIENTS RECEIVING INHALED CORTICOSTEROIDS

9.1 INTRODUCTION

As stated in the Chapter 1, inhaled corticosteroid therapy is considered to be first-line anti-inflammatory treatment for patients with persistent asthma[352]. However, many patients do not have adequate symptom control on low dose inhaled corticosteroids and additional second-line therapy is necessary. As all inhaled corticosteroids have been shown to be associated with dose-related adverse side effects, as illustrated in Chapters 3 to 5, there is increasing concern over the long-term risks of high dose therapy.

The results from Chapter 7 revealed the beneficial effects of a leukotriene receptor antagonist on disease control in patients with asthma and seasonal allergic rhinitis. Chapter 8 reported on the effects of a long-acting β_2 agonist when compared to inhaled corticosteroids in managing patient with asthma. It required to be seen, therefore, how these two forms of second-line therapy compare, in a head-to-head study, in patients not adequately controlled on inhaled corticosteroids. In other words, this study was designed to evaluate the best treatment option at stage 3 of the British Thoracic Society Guidelines[134]. This area is a contentious issue as the current asthma management guidelines were written prior to the introduction of leukotriene receptor antagonists and therefore there is no consensus of opinion.

The primary end-point was to assess effects on adenosine monophosphate bronchial challenge, however, other markers of inflammation (exhaled nitric oxide and blood eosinophil count) were assessed as well as symptom control, rescue therapy

requirements and lung function. As discussed in Chapter 1 (see section 1.5.1) long-acting β_2 agonist have been shown to exhibit desensitisation or tolerance. In order to investigate potential tolerance to the bronchoprotective effects on adenosine monophosphate challenge testing and lung function, these investigations were performed at the end of the first dosing interval (i.e. 24 hours after dosing with montelukast or 12 hours after dosing with salmeterol), and after two weeks of treatment. It is important to have a washout placebo phase, when patients use inhaled anticholinergic medication rather than β_2-agonist medication for rescue therapy, prior to this investigation in order to ensure receptor regeneration[275].

Montelukast and salmeterol were chosen, as they are the most commonly prescribed forms of medication in their class of therapy. Furthermore, the previous studies in this thesis have evaluated montelukast and it is licensed for once daily dosing which will aid compliance. Unfortunately, the previous study evaluated formoterol, in order to mirror the FACET study[62], and therefore no direct comparison can be made between this, and the previous study, in this thesis. However, the effects of formoterol and salmeterol are similar in terms of their clinical efficacy, their bronchoprotection and their propensity for down regulation[275].

9.2 METHODS

Patients

Twenty patients with moderate asthma (9 female), mean (SE) age 32.5 (2.2) years, FEV_1 79.1 (3.9) FEF_{25-75} 51.5 (4.5) % predicted were recruited into the study. All patients were required to be sub-optimally controlled despite taking more than 400μg per day of inhaled corticosteroids as monotherapy (median dose 800μg per day, inter-quartile range 400-1000μg per day) budesonide n=5 (800-1600μg per day); beclomethasone dipropionate n=14 (400-1000μg per day), fluticasone propionate n=1 (2000μg per day). Patients were eligible for inclusion if they required at least 2 puffs per day of reliever therapy with their usual short acting β_2 agonist at had at least 10% diurnal variability between their morning and evening peak expiratory flow rates. All patients were required to be responsive to adenosine monophosphate challenge testing with a provocation concentration producing 20% fall in FEV_1 (PC_{20}) of less than 200μg/dl (geometric mean 32.9 ± 9.5 mg/ml) prior to run-in period.

Design

The study was of a randomised placebo controlled, single-blind, double dummy, cross-over design. Patients continued on their usual maintenance dose of inhaled corticosteroid throughout the study. In addition, patients were randomised to receive the following: A) 50μg of inhaled salmeterol twice daily (as Serevent Accuhaler 50μg per actuation, Allen & Hanburys Ltd., Uxbridge, UK) plus placebo tablet once daily, or B) 10mg oral montelukast (as Singulair, Merck Sharp & Dohme Ltd., Herts UK) once daily plus

placebo Accuhaler twice daily. Prior to each treatment, and at cross over, patients had a one week treatment period with placebo Accuhaler (1 inhalation twice daily) and placebo tablets once daily while continuing with their inhaled corticosteroid. All tablets were taken at 0800hrs and inhaled medication was taken at 0800hrs and 2000hrs. Inhaled ipratropium bromide 2 puffs (Atrovent Forte, 40μg per puff, Boehringer Ingelheim, Bracknell UK) was used as required for symptomatic relief purposes as first-line rescue, with inhaled salbutamol (Ventolin, Allen & Hanburys Ltd., Uxbridge, UK) as second-line rescue.

Measurements

All laboratory measurements were performed at 0800hrs after the end of the one week run-in and crossover washout placebo periods, and after each two weeks active treatment periods. Patients also attended after the first dose of active therapy i.e. 12 hours after the first dose of inhaled salmeterol and 24 hours after the first dose of oral montelukast:

Adenosine Monophosphate Challenge Testing

Exhaled Nitric Oxide

Spirometry and Total Body Plesthysmography

Diary Card Data

Peripheral Blood Eosinophil Count

Statistical Analysis

The study was designed with at least 80% power to detect a 1.0 doubling dose difference (2.0 fold) in adenosine monophosphate PC_{20} (the primary end-point) with the alpha error

set at 0.05 (two-tailed). For all domiciliary diary data, mean values for the 7 day run-in and washout placebo periods and for the 2 weeks of each active treatment were analysed. Overall comparisons between active treatments and placebos were made by multifactorial analysis of variance (MANOVA) using subject, treatment, period and duration of treatment (first dose/last dose) as factors. This was followed by Bonferroni multiple range testing (set at 95% CI) in order to obviate multiple pair-wise comparisons. Consequently, comparisons are only denoted as being significant ($p < 0.05$) or not significant in order to not confound the alpha error.

9.3 RESULTS

There were no significant carryover effects between the first and second placebo values in sequence with any of the measurements [Table 9.1]. Consequently a pooled placebo value was used for the purposes of analysis.

AMP Challenge, Blood Eosinophil Count and Exhaled NO

For AMP PC_{20} (mg/ml) compared to placebo (47.5 ± 13.0), there were significant ($p<0.05$) differences with first (114.1 ± 36.9) and last (94.2 ± 30.4) doses of montelukast as well as the first (160.1 ± 64.5) but not the last (70.1 ± 23.7) dose of salmeterol [Figure 9.1]. Salmeterol, but not montelukast, was shown to exhibit significant ($p<0.05$) tolerance between the first and last dose protection against adenosine monophosphate bronchial challenge. This amounted to 1.19 doubling doses (95% CI 0.60 to 1.78) for salmeterol and 0.28 doubling doses (95% CI -0.31 to 0.85) for montelukast [Figure 9.2]. For blood eosinophil count after 2 weeks, there was a significant ($p<0.05$) difference between montelukast vs placebo; and montelukast vs salmeterol. (Placebo: 0.40 (0.06) $x10^9$/l, montelukast: 0.31 (0.04) $x10^9$/l, salmeterol: 0.44 (0.07) $x10^9$/l). There was no significant difference between either of the treatments and placebo in terms of exhaled nitric oxide after 2 weeks of treatment. (Placebo 10.5 (1.3) ppb; montelukast: 10.2 (1.5) ppb; salmeterol: 9.3 (1.6) ppb).

Lung Function

For forced expiratory volume in one second (FEV_1), forced mid expiratory flow (FEF_{25-75}), and specific airways conductance (sGaw), there was a significant ($p<0.05$) difference between placebo and the first, but not the last, dose of salmeterol. For montelukast there was no significant improvement except with the first dose in terms of FEF_{25-75} [Figure 9.3].

Domiciliary Diary Card Data [Table 9.2, Figure 9.4]

Salmeterol showed significant ($p<0.05$) improvements in terms of daytime and nighttime asthma symptom scoring and rescue bronchodilator requirement as well as morning peak expiratory flow rate. Montelukast showed significant ($p<0.05$) improvement in terms of nocturnal and daytime rescue bronchodilator requirement and morning peak expiratory flow rate.

Table 9.1

Means (SE) for first (run-in) and second (crossover) placebo washout periods in sequence for adenosine monophosphate bronchial challenge PC_{20} (AMP PC_{20}), exhaled nitric oxide (NO), peripheral blood eosinophil count (EOS), specific airways resistance (sGaw), forced expiratory volume in 1 second (FEV_1), forced mid expiratory flow (FEF_{25-75}), morning peak expiratory flow (PEFam), evening peak expiratory flow (PEFpm), daytime rescue bronchodilator requirement (RESam), night-time rescue bronchodilator requirement (RESpm), daytime symptom score (SYMam) and night-time symptom score (SYMpm). There were no significant differences for and points.

	First Placebo	Second Placebo		First Placebo	Second Placebo
AMP PC_{20} mg/ml	44.6 (12.4)	50.7 (15.8)	PEFam l/min	423.1 (17.5)	419.4 (15.5)
NO Ppb	10.3 (1.9)	10.8 (1.6)	PEFpm l/min	470.9 (18.3)	457.5 (17.4)
EOS $x10^9$	0.42 (0.06)	0.39 (0.06)	RESam puffs/12 hr	2.5 (0.4)	2.4 (0.4)
SGaw % pred	50.8 (6.9)	53.7 (7.6)	RESpm puffs/2 hrs	1.2 (0.3)	1.1 (0.3)
FEV_1 %pred	75.0 (3.4)	74.5 (3.4)	SYMam units/12 hrs	0.7 (0.1)	0.9 (0.2)
FEF_{25-75} %pred	45.4 (3.6)	45.5 (3.6)	SYMpm units/12hrs	0.6 (0.1)	0.6 (0.1)

Table 9.2

Means (SE) for domiciliary diary card data after 1 week of placebo (pooled), and after 2 weeks treatment of montelukast and salmeterol. Data are given for morning peak expiratory flow (PEFam), evening peak expiratory flow (PEFpm), daytime rescue bronchodilator requirement (RESam), night-time rescue bronchodilator requirement (RESpm), daytime symptom score (SYMam) and night-time symptom score (SYMpm). Asterisk denotes a significant difference between placebo and active treatments.

	Placebo	Montelukast	Salmeterol
PEFam l/min	426.3 (17.2)	433.5 * (16.6)	450.0 * (20.3)
PEFpm l/min	469.5 (18.5)	466.3 (17.8)	480.4 (19.9)
RESam Puffs/12 hr	2.41 (0.37)	1.54 * (0.31)	1.42 * (0.40)
RESpm Puffs/12 hr	1.14 (0.30)	0.76 * (0.21)	0.64 * (0.20)
SYMam Units/12 hr	0.81 (0.12)	0.61 (0.13)	0.47 * (0.11)
SYMpm unit/12 hr	0.58 (0.11)	0.46 (0.10)	0.41 * (0.11)

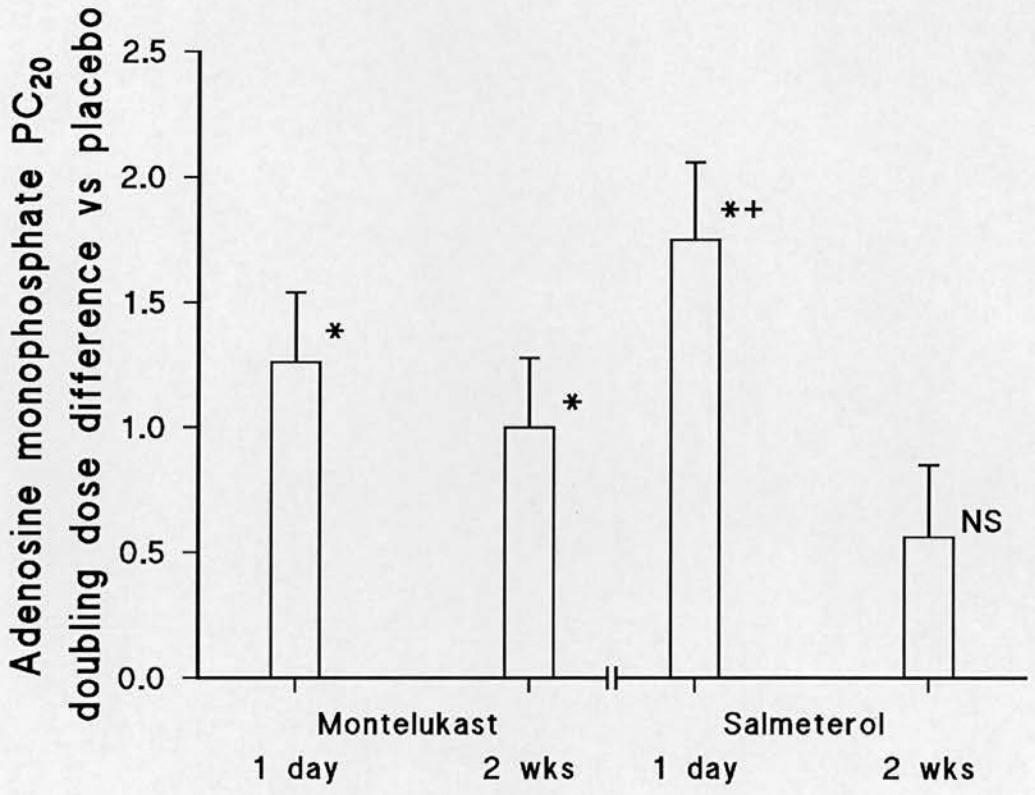


Figure 9.1
Means and SE for doubling dose difference from placebo (pooled) for adenosine monophosphate bronchial challenge provocation dose causing 20% fall in FEV_1 (PC_{20}) after the first dose (day 1) and last dose after 2 weeks therapy (2 wks) for montelukast and salmeterol. Asterisk denotes significant ($p<0.05$) difference from placebo. Cross denotes significant ($p<0.05$) difference between first and last doses of each drug.

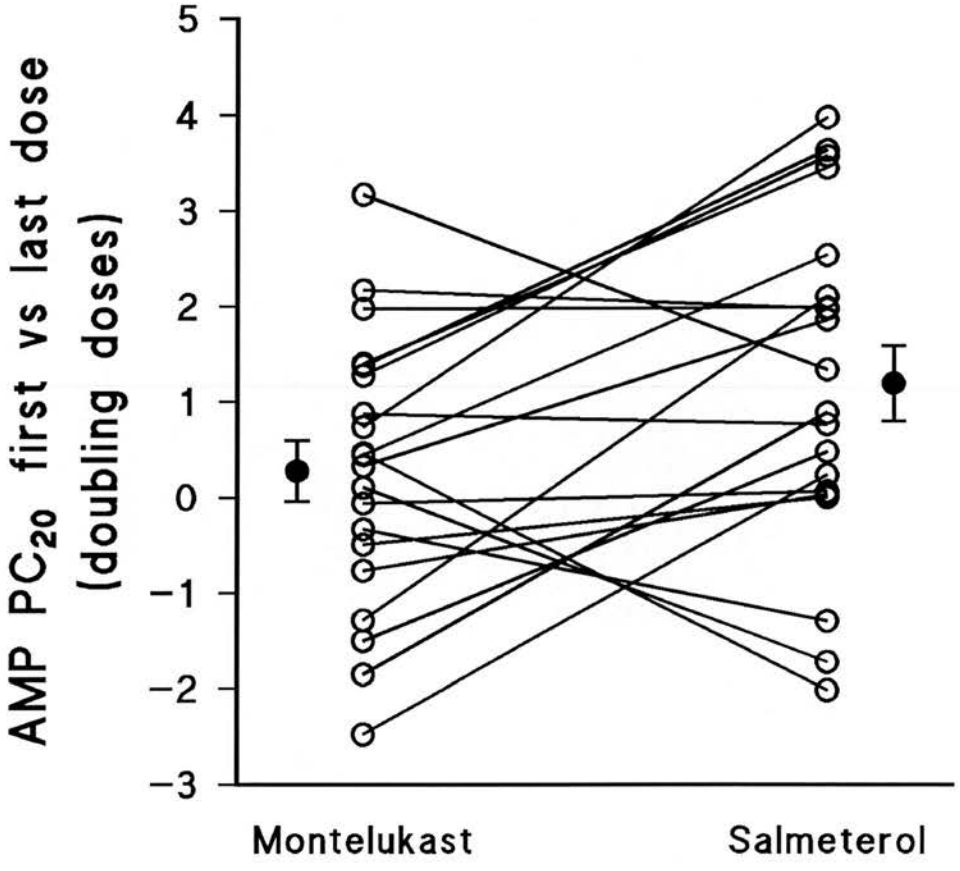


Figure 9.2
Scatter plot (open circles) for individual values and mean (SE) (closed circles) for loss of bronchoprotection against adenosine monophosphate between first dose and last dose as doubling dose difference for montelukast and salmeterol. There was significant ($p<0.05$) tolerance to bronchoprotection with salmeterol but not with montelukast.

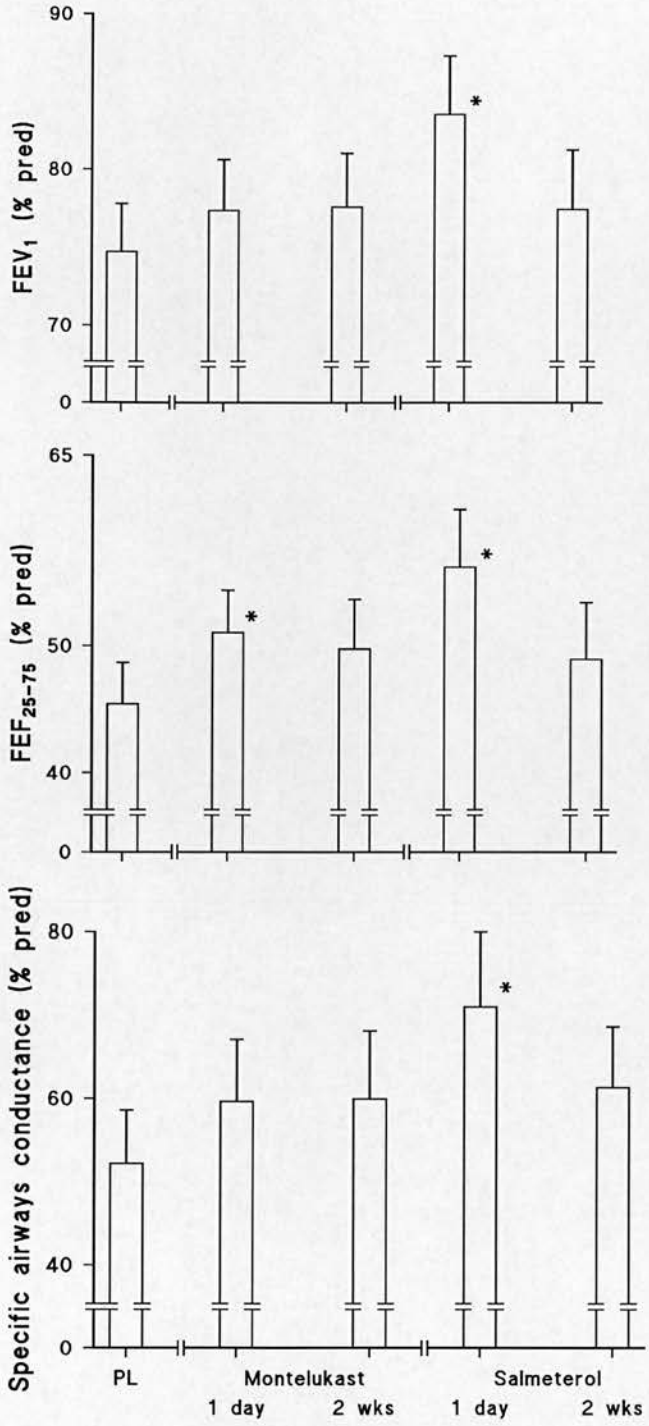


Figure 9.3
Means and SE for a) Forced expiratory volume in 1 second (FEV_1) b) Forced mid expiratory flow (FEF_{25-75}) c) Specific airways conductance as percent predicted for placebo (PL) and first dose (day 1) and last dose after 2 weeks therapy (2 wks) for montelukast and salmeterol. Asterisk denotes significant ($p<0.05$) difference between active treatment and placebo (pooled).

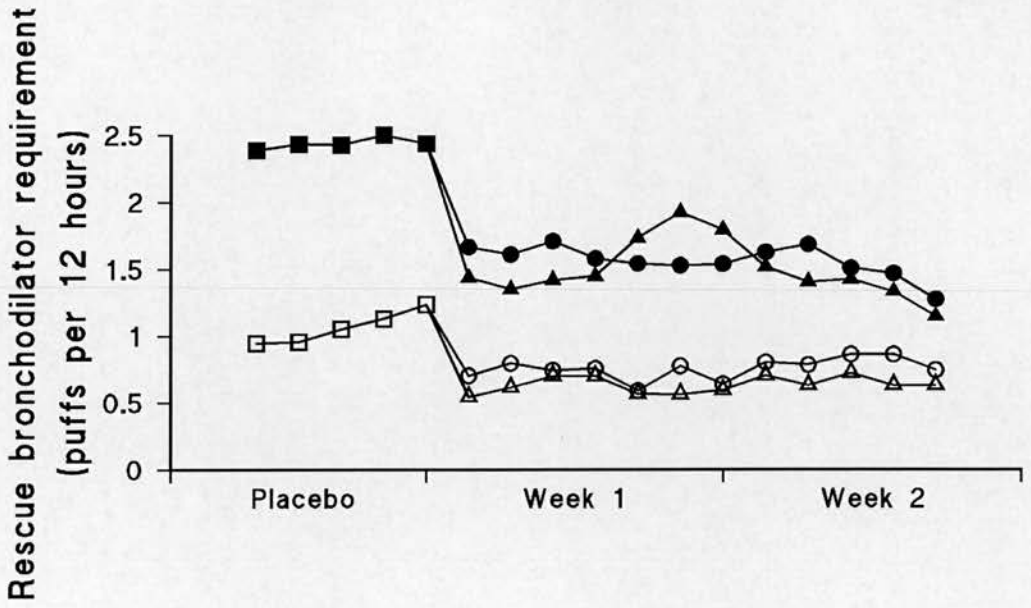


Figure 9.4
Three point moving average for daytime (closed symbols) and nighttime (open symbols) rescue bronchodilator requirement (puffs per 12 hours) for the 1 week placebo (pooled) (square), and the first and second week of active treatment with salmeterol (circles) and montelukast (triangles). There was a significant ($p<0.05$) difference for both drugs compared to placebo for both daytime and night-time requirements.

9.4 DISCUSSION

This is the first study to compare the effects of second-line therapy with a long-acting β_2 agonist or a leukotriene receptor antagonist, in patients not adequately controlled with inhaled corticosteroids, in terms of adenosine monophosphate bronchial challenge. It revealed a significant bronchoprotective effect after two weeks with the addition of once daily montelukast but not with twice daily salmeterol. This is in keeping with the study from Edellman et al[301], who found that, after 4 weeks of regular therapy, montelukast exhibited 57% protection against exercise induced bronchoconstriction compared to placebo whereas salmeterol exhibited 17% protection. Villaran et al[308] also compared montelukast and salmeterol in asthmatic patients, one quarter of which were taking inhaled corticosteroids, and showed greater beneficial effects of montelukast than salmeterol on exercise induced challenge.

In another study, salmeterol and zafirlukast have been compared in a 4 week parallel group study in which more than 80% of the patients were receiving concomitant inhaled corticosteroid therapy[300]. Both active treatments were associated with improvements from baseline in pulmonary function, asthma symptoms and short acting β2-agonist use. However, in contrast to the results in this chapter, salmeterol treatment resulted in significantly greater improvements from baseline compared with zafirlukast for morning peak expiratory flow (29.6 l/min vs 13.0 l/min), percentage of symptom free days (22.4% vs 8.8%) and percentage of days and nights with no requirement for supplemental short acting β_2-agonist use (30.5% vs 11.3%).

Previous studies have shown the benefit of adding a long acting β2 agonist to inhaled corticosteroids in terms of symptom control and exacerbation rates[62]. Leukotriene also exhibit additive benefit when given in combination to inhaled corticosteroids[299,384]. The extra effect of leukotriene receptor antagonist on top of inhaled corticosteroids can be partly explained by the poor suppression of leukotriene synthesis by inhaled corticosteroids. This was shown in study where fluticasone propionate reduced the response to allergen challenge but not the excretion of urinary leukotriene[385].

In this study, salmeterol was demonstrated to be a better bronchodilator than montelukast in terms of improvements in lung function in terms of spirometry after the first dose compared with placebo. However, there was no significant difference for either drug after two weeks of therapy compared to placebo. There was a significant difference between the first and last doses of salmeterol but not montelukast after adenosine monophosphate bronchial challenge, suggesting that tolerance to its effects had occurred. This is also in keeping with a study by Villaran et al[308] who showed tolerance to the effects of salmeterol after 4 weeks on exercise challenge.

There was no loss in the beneficial effects in terms of diurnal asthma control over the two week period (Figure 9.4) in contrast to marked loss in bronchoprotection against bronchial challenge (Figure 9.1). The apparent discrepancy between the comparative effects on bronchial challenge or lung function and diurnal asthma control may be explained by their relative degrees of tolerance. Tolerance with long-acting β_2-agonists

is recognised to be more pronounced with their bronchoprotective than bronchodilator effects[275]. Grove et al[386], showed in a study comparing the effects of salmeterol 50μg bid or placebo in patients taking inhaled corticosteroids, that although there were improvements in peak expiratory flow and reductions in rescue bronchodilator therapy, there was only a 0.7 doubling dose residual protection against histamine challenge after 4 weeks. In a study with regular formoterol 24 μg bid on top of inhaled corticosteroid therapy, there was 0.5 doubling dose protection against methacholine after 2 weeks with sustained improvement in peak flows[387]. Similarly with adenosine monophosphate bronchial challenge there was a 0.8 fold protection after 1 week of regular formoterol 24 μg bid[388].

There was a significant reduction in blood eosinophil numbers with montelukast but not with salmeterol, which is in keeping with previous studies[389,390]. However, it may not be valid to compare the effects of a topical drug, such as salmeterol, and a systemic drug, such as montelukast, on a systemic marker of disease activity. In contrast to the study in this chapter, recent data has shown that treatment with regular formoterol as mono-therapy to induce significant reductions in eosinophil numbers in bronchial biopsies[270].

Exhaled nitric oxide may be considered to be a sensitive marker of airway inflammation. However, there was no difference with either treatment when compared to placebo. This can be explained by data showing that the dose-response curve for exhaled nitric oxide

becomes flat after 400μg per day of inhaled budesonide[355]. As all of the patients studied here were receiving inhaled corticosteroids at a dose greater than 400μg per day prior to run-in, exhaled nitric oxide levels would not be expected to significantly change with the addition of second-line treatment.

It would have been more satisfactory if this study was performed in a double-blind fashion. However, the manufacturers of montelukast were not able to provide a matching placebo tablet. Although the tablets were masked, as far as possible, and put into separate bottles labeled only with code numbers, the tablets were not identical and patients could have been able to determine which tablet was the placebo treatment.

CHAPTER 10

GENERAL DISCUSSION

10.1 Overview

This thesis reports a series of pilot studies which explore aspects of anti-inflammatory medication used in the management of allergic airways disease. The dose-response characteristics for markers of the therapeutic and adverse effects of inhaled corticosteroids were examined. Other disease modifying therapies were also assessed, namely leukotriene receptor antagonists and long-acting β_2-agonists, in terms of their anti-inflammatory properties, and comparisons were made between these drugs and inhaled corticosteroids.

10.2 Dose-response for systemic activity of inhaled corticosteroids

The studies in the first chapters investigated the systemic effects of inhaled and intra-nasal corticosteroids. By comparing the effects of two inhaled corticosteroids with different pharmacological properties, it was shown that the combination of greater topical potency, and blood and tissue accumulation potentates more severe systemic effects (Chapter 3 – Study 1). However, when assessing the systemic activity of two inhaled corticosteroids of similar potency there was no difference between the drugs (Chapter 3 – Study 2). Therefore increasing the potency of a drug will not only increase the clinical efficacy but also the adverse effects[391].

When the first studies were performed, the most widely used inhaled corticosteroids in the United States of America were triamcinolone acetonide and flunisolide. Fluticasone propionate had just received a license for use in USA. The first two studies therefore had

important clinical implications for an American audience, as there were no dose-response studies comparing triamcinolone acetonide with fluticasone propionate or flunisolide using sensitive measures of adrenal function. The reason for performing dose-response studies has been discussed previously (see sections 1.4.1 and 3.1), namely the ability to determine whether comparisons are made on the flat or steep part of the dose-response curve.

Evidence from these studies (Chapters 3, 4 and 5), i.e. inhaled corticosteroids cause adverse systemic activity at high doses, is in keeping with other data[124,177,392]. However this is in contrast studies which had reported that fluticasone propionate causes no adrenal suppression[179,180]. The reason for this discrepancy may lie in the sensitivity of the measures of adrenal function, as measurement of morning serum cortisol is less sensitive than measurement of overnight urinary cortisol (see section 1.3.4).

Some consideration, therefore, needs to be taken regarding sensitivity of the measures of systemic activity. In the first two studies in Chapter 3 there were no significant difference between triamcinolone acetonide and placebo at 1600μg per day in terms of 8am serum cortisol, but a significant difference in terms of overnight urinary cortisol/creatinine ratio. It is therefore clearly possible to incorrectly report that a particular inhaled corticosteroid has no detectable systemic bioactivity if an insensitive test is used. In this respect some studies have concluded no significant systemic bioactivity of intra-nasal corticosteroids by using the 6 hour 250μg ACTH stimulation

test[238], although there may have been detectable suppression if a more sensitive test had been used.

The low dose (0.5μg) ACTH stimulation test is thought to be more sensitive that the standard (250μg) ACTH test[188], and comparable to the unpleasant insulin tolerance test[187]. However, there was a lack of response with inhaled (Chapter 3) or intra-nasal (Chapter 5) corticosteroids using the low dose ACTH test, despite changes in urinary cortisol. This may be explained by the relatively short duration of treatment. The effects on dynamic testing are thought to take longer to occur than basal measures of adrenal function, as the former reflects adrenal atrophy. Indeed, in a study by O'Driscoll et al, oral corticosteroids were stopped abruptly after a short period of treatment without any evidence of rebound worsening of asthma and or adrenal crisis[393]. However, Clark et al[323] found that high dose inhaled corticosteroids attenuated the cortisol response to corticotropin stimulation after 3 days. Recently there has been concern regarding the low dose ACTH stimulation test as a lack of response may be due to absorption of the drug onto the surface of a plastic syringe or tubing used in administration[394].

The second study in Chapter 3 and the last study in Chapter 5 both highlight the sensitivities of fractionated samples of urinary cortisol/creatinine. In Chapter 3, morning and overnight urinary cortisol measurements were more sensitive than morning serum cortisol and in Chapter 5, these measurements were shown to be as sensitive as integrated 24 hour serum and urine cortisol measures. MacIntyre et al[185] showed that

timed morning and overnight samples of urine cortisol/creatinine were as sensitive as a full 24 hour urinary free cortisol collection at detecting changes in adrenal suppression in patients receiving 2mg per day of beclomethasone dipropionate. The reason for the greater sensitivity of an early morning urinary cortisol/creatinine sample is probably because the collection reflects the time in the circadian cycle when cortisol secretion is at its highest (see Figures 5.6 and 5.9), and therefore giving the opportunity of high signal to noise ratio. An early morning urine collection taken immediately on wakening would also avoid the need for accurate timing, and it would not be difficult for patients to collect and bring such a sample to an outpatient clinic for analysis. With further work assessing patient variability and reliability, a sample of urine cortisol/creatinine collected during the first hour of wakening may be a prove to be a useful screening test.

The findings with triamcinolone acetonide in the first study in Chapter 3 are in contrast to those of the last study in Chapter 5. In Chapter 3 there was no significant suppression of overnight urinary or serum cortisol compared to placebo at 1600μg per day, whereas significant suppression was detected in Chapter 5. Both studies employed asthmatic patients of mild to moderate severity and the duration of treatment was similar (5 days vs 3 days at the high dose). The discrepancy may be due to the fact that the third study in Chapter 5 had supervised dosing and patients were admitted for 12 hours prior to the 8am serum cortisol sample. By admitting the patients to hospital, it was possible to ensure that the overnight urine collection was complete and that conditions were standardised prior to morning serum cortisol sampling. However, when analysing the

data in terms of the number of patients with sub-normal values, the results of the two studies were identical. There were 3/24 patients with an abnormal overnight urinary cortisol suppression in Chapter 3 and 3/24 and 4/24 patients with a subnormal 8am serum cortisol and 24 hour urinary cortisol in Chapter 5 (see Figure 3.2 and Figure 5.10). Furthermore, the results from the third study in Chapter 5 are in keeping with the main conclusions from the first study in Chapter 3 as there was a two-fold difference between fluticasone propionate and triamcinolone acetonide with overnight urinary and 8am serum cortisol.

On first inspection it would seem that there is a discrepancy between Chapter 6 and Chapter 8 in terms of cortisol suppression with budesonide. In the study comparing budesonide with formoterol (Chapter 8) there was no suppression with either 400μg or 800μg per day of inhaled budesonide. However in Chapter 6 a dose-repose effect was found for systemic activity of budesonide over a dose range of 400-1600μg per day. In both studies patients with mild to moderate asthma were investigated and budesonide was dispensed using a Turbuhaler. However, in Chapter 8 budesonide was given once daily whereas in Chapter 6 it was given twice daily. It is has been suggested that the suppressive effects of corticosteroids on diurnal endogenous cortisol production are less when given as a once daily morning (or alternate day) dose compared to twice daily or nocturnal dosing for a given level of clinical efficacy[235].

10.3 Effect of inhaler device and drug delivery

It is important to consider, not only the particular inhaled corticosteroid and dose prescribed to the patient, but also the drug delivery device. This is becoming increasingly clinically relevant due to the expanding number of different inhaler devices available. It is evident from the results shown in Chapter 3, that the inhaler device plays an important role in the determination of adverse effects. Changing the delivery of fluticasone propionate from an Accuhaler to a pressurised metered dose inhaler plus spacer resulted in a 7.68-fold difference in overnight urinary cortisol/creatinine suppression. This is much greater than was seen when comparing fluticasone propionate and triamcinolone acetonide both via metered dose inhalers (1.9 fold difference). Previous work has shown a spacer device decreases the systemic effects of beclomethasone dipropionate[395] although this was probably due to a decrease in the swallowed fraction of the inhaled drug. The influence of the swallowed fraction on systemic bioactivity is much less with modern inhaled corticosteroids as a result of the high first-pass hepatic metabolism.

The last study in Chapter 3, however, only investigated the influence of drug delivery on the adverse profile in terms of systemic effects. However, it would be interesting to investigate whether the delivery device would influence the beneficial effects and therapeutic ratio of inhaled corticosteroids. In this respect the increased delivery with a spacer has been shown improve the therapeutic ratio with budesonide[171]. If an inhaler delivers a drug with a low respirable fraction, the drug will be mainly deposited in the

proximal airways[396,397]. This is unlikely to have a beneficial effect on the small airways which are effected in asthma, but is also may also exhibit less systemic absorption. Further research requires to be performed to investigate the effects of the site of drug delivery on systemic bioactivity, clinical efficacy and therapeutic ratio.

10.4 Comparison of inhaled and oral corticosteroids

Although there is little doubt that inhaled corticosteroids exhibit a greater therapeutic ratio than oral corticosteroids, there is still useful clinical information to be gained from comparing these two forms of therapy. Both practitioners and patients can identify with the effects of oral corticosteroids. Some patients are aware that ingesting a tablet may result in systemic effects but are unaware that inhaling a gas may do likewise. There were no significant differences between the effect of prednisolone and fluticasone propionate when compared on an 11:1 dose ratio. This is in keeping with a meta-analysis of dose-response studies[398] which showed, when assessing the effects on 8 or 9am serum/plasma cortisol, fluticasone propionate was shown to have equivalent suppression to prednisolone on a 10:1 milligram equivalent ratio.

When analysing the results of the study in Chapter 4, comparable adrenal suppression was seen with oral prednisolone and fluticasone propionate but there was no significant dose related suppression with nebulised budesonide up to a dose of 4mg per day. The reason for the finding with nebulised budesonide is probably due to the inefficient delivery characteristics of nebulisers as discussed above (see section 4.4). It is

interesting to note the reproducibility of 8am serum cortisol as a marker of systemic effects of corticosteroids when the variability of lung delivery is removed by prescribing this drug in tablet form. There was 67% and 69% suppression of plasma cortisol with 20mg of oral prednisolone in the first and second studies respectively. Furthermore, when analysing the numbers of cortisol samples below the normal reference range (150nmol/l) there were 42% and 36% in the two studies. The consistent systemic bioavailability (as well as dose) may account for the relatively uniform improvement with steroid responsive patients taking oral corticosteroids.

The results in Chapter 4 suggest that there are different effects of inhaled corticosteroids on different tissues. There was a significant difference between placebo and all doses with both inhaled fluticasone propionate and oral prednisolone in terms of 8am cortisol whereas for osteocalcin there was only suppression at the highest dose with fluticasone propionate and the medium and high doses for osteocalcin. Although it may simply represent the relative sensitivities of the tissue markers, studies have shown differential effects on adrenal and bone function when comparing inhaled budesonide and oral prednisolone[208,209]. It could therefore have a clinically important implication if, for a given change on adrenal function, bone metabolism (and therefore potential for osteoporosis) is less effected. Regardless, it clearly indicates that screening for changes in bone mass with bone densitometry is important and cannot be replaced by simple screening for changes in adrenal function because the effects are not the same on different tissues.

10.5 Comparisons of intra-nasal corticosteroids

Similar findings for inhaled corticosteroids hold true when comparing therapeutic doses of different intra-nasal corticosteroids (Chapter 5). There was no systemic bioactivity with weakly potent corticosteroids, beclomethasone dipropionate and triamcinolone acetonide, but treatment with fluticasone propionate resulted in significant suppression in terms of overnight urinary cortisol. Increased potency is clearly not the only reason for detectable side effects with intra-nasal corticosteroids, as mometasone furoate, which has equivalent potency to fluticasone propionate, did not exhibit any significant suppression. The second study in Chapter 5 had greater statistical power than the second study in that chapter, and utilised more sensitive measures of basal adrenal function namely 24 integrated serum and urinary cortisol[175]. The reason for the lack of systemic effects seen in the second study in Chapter 5 could be that patients with allergic rhinitis were investigated (the first study evaluated healthy volunteers) and this is likely to have altered the delivery to the nose.

Allergic rhinitis and asthma may be considered to be two clinical manifestations of the same condition[37]. As a result, patients may receive both inhaled and intra-nasal corticosteroids simultaneously. Furthermore, patients with allergic asthma often need to increase their dose of inhaled corticosteroid during the summer at the same time as introducing intra-nasal corticosteroid due to increased pollen counts. Although the effects on systemic activity of intra-nasal flunisolide in patients receiving inhaled corticosteroids has been investigated in one study[399], the addition of combined inhaled

and intra-nasal therapy is often ignored. The last study in Chapter 5 addresses this issue, and the results are in keeping with the first studies in Chapters 3 and 5, as the addition of intra-nasal to inhaled fluticasone propionate resulted in more patients having a subnormal (<150nmol/l) 8am serum cortisol value. Further studies are needed to investigate the effects of the total steroid load when treating patients with seasonal allergic rhinitis and asthma.

In all of the above studies the results have been expressed as both mean values and number of values below a clinically relevant range. By assessing the number of abnormal values, it is possible to address the clinical issue of whether a given patient is likely to have clinically significant systemic adverse effects with a particular inhaled corticosteroid. Furthermore, the data from Chapters 3, 5 and 6 show that patients with low basal cortisol values are more likely to have a subnormal response to HPA-axis stimulation, which is in keeping with Broide et al[188]. It can be seen by examining the scatter plots from the studies in this thesis (Figures 3.2, 3.4, 3.6, 4.2, 4.4, 5.5, 5.10), that there is a great deal of patient variation in response to inhaled corticosteroids. Further studies are required to investigate any genetic susceptibility to exaggerated adverse effects with corticosteroid therapy.

10.6 Limitations of the systemic effect studies in this thesis

There are limitations to all of the efficacy studies performed in Chapters 3, 4 and 5. All of the studies were of short duration. Indeed most of the dosing intervals were only for 3

or 4 days. However, the effect of exogenous corticosteroids on endogenous HPA-axis activity is related to plasma drug levels. Therefore once drug levels have reached steady state, prolonging the duration of the study is unlikely to significantly alter the results. As fluticasone propionate has the longest elimination half-life it will take the longest time to reach steady state. However, even with fluticasone propionate, constant serum levels will be achieved within 3 days.

There is, however, evidence of tolerance to inhaled corticosteroids measured in terms of glucocorticoid receptor down regulation. As shown by Knutsson et al[237], there was a significant down regulation in peripheral lymphocyte glucocorticoid receptor (GCR) messenger ribonucleic acid (mRNA) after 2 weeks of intra-nasal fluticasone propionate. More recently, Andersson et al[400] showed 30% suppression of endobronchial biopsy GCR mRNA and 70% suppression of lymphocyte CGR mRNA after 500μg fluticasone propionate for 4 weeks. However, in a study by Altman et al[236], the effects of triamcinolone acetonide on 24 hour urinary cortisol were similar after 2 weeks and 6 months, therefore glucocorticoid receptor down regulation does not seem to be clinically relevant when assessing the effects of inhaled corticosteroids.

For the same reasons it may be considered that the placebo washout periods may also be too short. Indeed Knutsson et al[237] also showed that the effects of fluticasone propionate on GCR mRNA persisted for more than 1 week. However, in another study it was shown that the effects of fluticasone propionate on basal and dynamic measures of

HPA-axis activity returned to baseline after 3 days of placebo washout[401], which was the minimum washout period in all of the studies in this thesis.

Another concern may be that the patients studied often had mild disease, and would not be receiving the highest doses of inhaled corticosteroids investigated in the dose ranging studies. In this respect airway calibre has been shown to be related to the systemic bioavailability and therefore the adverse effects of drugs[402]. In other words, patients with more severe asthma will have smaller airway calibre and therefore less systemic absorption resulting from poorer lung delivery. Indeed Weiner et al[403] showed a significant correlation between FEV_1 and fall in nocturnal cortisol concentration in patients receiving 500μg fluticasone propionate. For this reason in all of the adverse effect studies (Chapters 3 and 4), spirometry was measured in order to ensure that changes in systemic effects were not due to alterations in lung delivery.

Two studies, published as abstracts, show that the systemic activity of fluticasone propionate is less in asthmatic patients than healthy volunteers[404,405]. However, the relative effects of comparing two different inhaled corticosteroids, in healthy volunteers as opposed to patients, are not established. Lofdahl & Thorsson[406] showed no difference between these two subject groups when comparing fluticasone propionate and budesonide in terms of plasma drug concentrations. However, Harrison et al[407], showed greater systemic activity between fluticasone propionate and budesonide in healthy volunteers but not in patients with asthma, in terms of cortisol suppression. Although

there may be differences in mucous production, mucocillary clearance and absorption of drugs, it is likely that the difference in airway calibre is the major confounding factor.

In all of the studies, except for the comparisons with prednisolone in Chapter 4 where potency ratios were calculated from previous data, the drugs were compared on microgram equivalent dosing rather than on therapeutically equivalent doses. However, there were no *in vivo* dose-response studies comparing the relative potency of inhaled triamcinolone acetonide with fluticasone propionate or flunisolide which could be used to give potency ratios. In one study, Condemi et al[408] compared triamcinolone acetonide 200μg four times per day with fluticasone propionate 250μg twice daily and showed that the lower daily dose of fluticasone propionate resulted in greater symptom control. However, it is known that four times per day dosing results in poorer compliance than twice daily dosing and this may have influenced the results, as compliance was not measured during the study. As discussed previously (see section 1.3.2), it is not possible to accurately calculated dosing ratios by using potency data from *in vitro* assays or from the McKenzie vasoconstrictor assay. It seemed logical therefore to compare drugs on a microgram equivalent basis until further information is available. This is particularly the case as practitioners often change patients from one inhaled corticosteroid to another on microgram equivalent basis. However, in light of the results from the first study further dose-response studies should be performed with higher doses of triamcinolone acetonide in order to produce parallel dose-response curves and determine a potency ratio.

In all of the studies, apart from the third in the first paragraph and the nasal studies, the treatments were given sequentially in increasing doses with no washout period in between. This means that the duration of treatment as well as dose may have affected the response. However, each treatment period was given to allow steady state levels, and doses were given in sequentially doubling increments. Therefore it is likely that the dose, rather than duration of treatment, had the major effect on outcome measures.

Ideally the doses should have been given in a random order with a washout period in between. Alternatively a parallel study design could have been employed which would have required the same number of patient groups as the product of the drugs and doses to be compared. In other words when comparing fluticasone propionate and triamcinolone acetonide in the first study in Chapter 3, there were two drugs, each given at three different doses, requiring 6 patient groups in a parallel study. The first method would have increased the duration of the study and the second would have considerably increased the number of participants required to complete the study. Both of these designs would have made the study more difficult to complete and more expensive to perform. In this respect, it is important to consider the implication of resources and cost, when designing a study.

10.7 Clinical relevance of systemic effect studies

What is the clinical relevance of these studies? Brown et al[184] showed that 20% of patients receiving high dose inhaled corticosteroids had abnormalities of HPA-axis

activity, which was mostly related to the duration of dose and previous oral corticosteroids. The incidence of adrenal crisis as a result of inhaled corticosteroid usage is extremely rare. However, there are case reports of clinically relevant adrenal suppression with fluticasone propionate. A 50 year old woman presented with Cushingoid features, depressive psychosis, adrenal suppression, osteopenia, and hypertension after receiving 2mg per day of inhaled fluticasone propionate. Her appearance, mental state and adrenal function returned to normal after her asthma therapy was changed to inhaled budesonide 0.8mg per day, without deterioration in her lung function[409]. Todd et al[410] have also reported on a 6 year girl with acute adrenal insufficiency after changing from inhaled fluticasone propionate 1mg per day to budesonide 0.8mg per day. Other authors have reported cases of adrenal suppression with fluticasone propionate[411]. Addisonian crises have been seen on withdrawal of budesonide, previously given at a high dose of 4.8 mg per day, in a 38 year old man[192], and beclomethasone dipropionate, previously given at a dose of 400μg per day, in a 7 year old girl[193].

As previously discussed (see section 1.4.3.2) changes in serum markers of bone metabolism may not be able to predict long term effects on bone mineral density and osteoporosis. However, reduction in bone density is an important clinically adverse effect of inhaled corticosteroids, which has been shown to be related to dose[203]. Indeed Wong et al[412] showed a 0.16 standard deviation reduction in bone density per doubling dose of inhaled corticosteroids. McEvoy et al[413] reported an odds ratio of vertebral

fraction in male asthmatics of taking inhaled corticosteroids of 1.35 compared to patients not treated with inhaled corticosteroids. Patients, particularly post menopausal women, receiving high dose inhaled corticosteroids should therefore take measures to reduce the chances of osteoporosis, for example smoking cessation, physical activity, calcium supplementation and hormone replacement therapy[201,414].

10.8 Dose-response for clinical efficacy

Just as there are differences in the sensitivities of markers of adverse effects of inhaled corticosteroids, there are differences in the shape of the dose-response curve for measures of efficacy. Although it is realised that the dose which relieves patients' symptoms may not normalise their spirometry, the study in Chapter 7 examines the dose-response effects on efficacy markers in more detail. An important clinical message from this study is that the dose required to achieve spirometric control did not necessarily produce adequate suppression of surrogate markers of airways inflammation. As discussed in Chapter 7, this is in keeping with the finding of inflammation in pre-symptomatic patients not receiving treatment. Monitoring steroid dose by spirometry may miss underlying airways inflammation and potentially lead to long-term fixed airways obstruction.

The clinical implication of the findings of the study in Chapter 6 is illustrated in a 2 year prospective study[415]. All 75 patients had measurement of methacholine bronchial challenge testing, as well as spirometry and asthma symptoms. Patients were randomised

to have their steroid dose adjusted according to a standardised computerised algorithm using either symptoms and spirometry, or with the addition of airway hyperresponsiveness data. After 2 years it was shown that by using bronchial hyperresponsiveness data, not only did the patients have better spirometry values, but they had fewer exacerbations. Mild exacerbations occurred at a rate of 0.52 per patient per year in those who did not increase their inhaled corticosteroids dose, but met the bronchial hyperreactivity criteria for doing so. Indeed patients who had severe asthma and were treated according to hyperresponsiveness protocol had the same outcome as patients with mild asthma, whereas severe patients monitored according to symptoms had a significantly poorer outcome. More importantly, patients monitored according to methacholine challenge had a greater improvement in bronchial airway thickness at the end of 2 years, when bronchial biopsies were compared with enrolment samples.

These findings are probably a result of treating asymptomatic airway inflammation which can be detected by methacholine bronchial challenge as shown in Chapter 6. The more aggressive treatment of inflammation, however, resulted in a higher prescribed dose of inhaled corticosteroids. The average dose was 400μg per day in the standard group and 800μg per day in the group monitored according to hyperresponsiveness. As no measure was made of systemic effects, the benefit in terms of therapeutic ratio cannot be determined. It also shows the importance of prescribing anti-inflammatory second-line therapy and the need for the studies in Chapters 7-9.

10.9 Measurements of clinical efficacy

Measurement of nasal and exhaled nitric oxide has been regarded as sensitive, non-invasive indicators of upper and lower airways inflammation respectively. Nitric oxide levels have been shown to change in relation to corticosteroid therapy[369] and correlate closely with other markers of inflammation such as induced sputum[105] and blood eosinophilia[416]. The results from Chapter 7 illustrate the sensitivity of this marker. Significant suppression of both exhaled and nasal nitric oxide in patients with asthma and rhinitis was achieved by 400μg of inhaled budesonide and 200μg of intra-nasal budesonide. Interestingly, in that study (Chapter 7), detectable suppression in exhaled nitric oxide was seen after treatment with montelukast, which is in keeping with its anti-inflammatory properties. The results from the dose-response study in Chapter 6 are in keeping with those of Jatakanon et al[355], who also found that, in mild to moderate patients, the values for exhaled nitric oxide normalised at low dose (400μg per day) with no further suppression at higher doses.

The results from Chapters 8 and 9 also support this finding. In Chapter 8, there was significant suppression of nitric oxide with 400μg per day budesonide compared to placebo, which amounted to a 1.9 fold difference. However, doubling the dose of budesonide to 800μg per day resulted in no further suppression and indeed a similar difference from placebo (2.1 fold difference). The results of the study comparing the anti-inflammatory effects of montelukast and salmeterol (Chapter 9) were initially surprising as there was no suppression of exhaled nitric oxide after treatment with the

leukotriene receptor antagonist. This seemed to be in contrast to the study in Chapter 7. However, it can be explained by the fact that all the patients were receiving inhaled corticosteroids at a dose greater than 400μg per day and would have already suppressed their exhaled nitric oxide to a low level. Thus exhaled nitric oxide may be useful in monitoring the disease activity in mild patients but is of less value when comparing the anti-inflammatory properties of second-line agents.

The different stimuli for a bronchial challenge testing have different advantages and disadvantages. In keeping with other authors[360] adenosine monophosphate was found to be more sensitive than direct bronchial challenge with methacholine in the dose-response study in Chapter 6. Other authors have also shown adenosine monophosphate challenge to be more specific in terms of diagnosing asthma from other chronic airway diseases in children[417]. This is probably because it does not exert its bronchoconstrictive effects by acting on smooth muscle (as does methacholine and histamine) but acts as an indirect stimulus like allergen, cold air or exercise. It is, therefore, considered to be more representative of real-life asthmatic responses[79]. In this respect, adenosine monophosphate bronchial challenge has been shown to cause more discomfort in terms of "chest tightness" than methacholine[418]. This is probably due to the action of mediators on airways sensory nerves. It can also be seen from Figure 6.2 that the adenosine monophosphate dose-response curve is steep for mild to moderate asthmatics between a dose of 400μg and 1600μg per day whereas methacholine is flatter until 800μg per day.

Adenosine monophosphate bronchial challenge was also shown to be reproducible in the studies in this thesis. When comparing the effects of inhaled budesonide, as a doubling dose difference from placebo, from all the relevant studies, a similar response was found. There was a 2.67 doubling dose difference with 400μg inhaled budesonide in the dose-response study (Chapter 6), a 2.67 doubling dose difference in the study comparing budesonide (400μg per day) and montelukast (Chapter 7) and a 2.46 doubling dose difference with low dose budesonide (400μg per day) in the study comparing budesonide and formoterol (Chapter 8). This may have been because the patients were all of mild to moderate severity and had a similar baseline adenosine monophosphate PC_{20} after placebo in each study. Also of interest is the similar clinical efficacy achieved by taking budesonide 400μg per day, as a once daily dose (Chapters 7 and 8) compared to two divided doses (Chapter 6). Indeed, budesonide is licensed for once daily usage at this dose.

However, there are problems when interpreting the results of bronchial challenge testing in studies comparing different classes of anti-inflammatory therapy for asthma. As stated above (see section 1.2.2), adenosine monophosphate acts by binding to adenosine receptors on mast cells causing them to degranulate and release inflammatory mediators inducing bronchoconstriction. Of these mediators, the most important are histamine, prostaglandins and leukotrienes[419]. Therefore it may be possible to block the effects of adenosine monophosphate, without controlling airway inflammation, with a leukotriene

receptor antagonist (as in Chapter 7) or an anti-histamine[420] or their combination[283]. However, montelukast also suppressed exhaled nitric oxide suggesting an anti-inflammatory property. This highlights the importance of performing more than one measurement of disease control and taking a global picture from the results.

There were significant improvements in bronchoprotection with inhaled short-acting (Figure 6.2) and long-acting β_2-agonists (Chapter 9 and 10). The effects of β_2-agonists on bronchial challenge are probably due to functional antagonism as lesser effects are seen with other markers of inflammation. Although the adenosine monophosphate bronchial challenge has been used to study the effects of cromones[421], data evaluating the effects of other anti-inflammatory medication on this challenge are sparse.

Evaluations of simple breathing tests and patients' symptoms are often used to monitor disease activity in asthmatic patients. In Chapter 6, patients' symptoms and lung function achieved their maximum response at a moderately low dose (400μg per day) of inhaled budesonide, probably as their disease was of mild to moderate severity. Measurements of spirometry performed at a clinic are relatively insensitive and therefore large numbers of patients are required to make statistical conclusions. Not surprisingly, therefore, there were no differences between any of the active treatments by using these measures and only in one study was active treatment statistically different from placebo (Chapter 8). Daily peak flow measurements are more sensitive as, even with the small sample sizes in this thesis, it was possible to detect a difference between the combination

of budesonide plus formoterol and either treatment as monotherapy (Chapter 8), and an improvement with montelukast or salmeterol when compared to placebo in Chapter 9. This shows the importance of providing asthmatic patients with a peak flow meter as this cheap and simple method of monitoring asthma is not only sensitive but allows patients to be in control of monitoring their own disease.

10.10 Second-line anti-inflammatory therapy in allergic airways disease

Increasing the dose of an inhaled corticosteroid has greater control of airway inflammation (Chapter 6), although it also results in more systemic adverse effects (Chapters 3,4,5). In this respect in Chapter 6, the therapeutic index was shown to be poorer at the highest dose. Although there were more patients with a clinically significant decrease in bronchial hyperresponsiveness there were also more patients with sub-normal urinary cortisol levels. The lowest possible maintenance should therefore be prescribed in keeping with current asthma management guidelines[134].

Further control of inflammation requires second-line treatment including long-acting β_2 adrenoceptor agonists and leukotriene receptor antagonists. As discussed previously (see section 1.5.1), there is currently a debate as to whether long-acting β_2 agonists have anti-inflammatory properties or not. The results from the study designed to investigate this further (Chapter 8) suggest that the beneficial effects of formoterol are not due to controlling airway inflammation as there were no significant effects on exhaled nitric oxide, adenosine monophosphate, bronchial challenge, or eosinophilic cationic protein.

Furthermore, in terms of effects with salmeterol (Chapter 9), there were also no improvements in eosinophilic cationic protein or bronchial challenge testing after repeated dosing.

There is no doubt that long-acting β_2-agonists improve lung function as can be seen in both of these studies. Also patients seemed to prefer this form of treatment rather than budesonide as monotherapy (Chapter 8). Given their reported benefits on exacerbation rates and quality of life[62], long-acting β_2-agonists have an important role in the treatment of asthma. They are especially useful in achieving rapid control of brittle asthmatics while other anti-inflammatory therapies take effect. Now that they are becoming available in combination with inhaled corticosteroids in the one inhaler device they may even increase the compliance with inhaled corticosteroids.

Leukotriene receptor antagonists are a new form of anti-inflammatory therapy available for allergic patients. The results from the studies in this thesis suggest that they may have an important role in both asthma, as first-line (Chapter 7) or second-line (Chapter 9), as well allergic rhinitis (Chapter 7). Montelukast offered symptomatic control of allergic rhinitis, although there were significantly greater effects on nasal nitric oxide and nasal peak inspiratory flow rate with intra-nasal budesonide. Similarly inhaled budesonide improved bronchial hyperreactivity to a significantly greater effect than montelukast, reflecting the high degree of anti-inflammatory activity of inhaled corticosteroids[134]. In a head-to-head comparison with a long-acting β_2 agonist

(salmeterol), montelukast achieved equivalent control of symptoms and lung function and seemed to have a greater effect on the inflammatory markers of eosinophilic cationic protein and adenosine monophosphate bronchial challenge (Chapter 9).

It is also clear from Chapter 9, that the effects of leukotriene receptor antagonists occur as quickly as long-acting β_2 agonists (Figure 9.4). A significant improvement in lung function and bronchial challenge testing occurred within 24 hour for both treatments. This is obviously more rapid than the effects of inhaled corticosteroids which showed no significant improvements in lung function after 1 week of treatment in the studies assessing systemic effects (Chapters 3). It may be due to the bronchodilatory properties of leukotriene receptor antagonists as they inhibit the very potent bronchoconstrictor leukotriene D4[422]. However, it does mean that there is potential for gaining patients' confidence quickly. As discussed previously (see section 1.5.1) long-acting β_2-agonists exhibit tolerance to their therapeutic effects although the clinical importance of this is uncertain. However, there was no evidence of tachyphylaxis with montelukast in terms of spirometry or adenosine monophosphate bronchial challenge (Chapter 9).

10.11 Limitations of efficacy studies in this thesis

A weakness of the efficacy studies is the short duration of treatment. Although Kraan et al[251] showed that there was no significant increase in bronchoprotection against histamine challenge test with 800μg per day of budesonide after 8 weeks compared to 2 weeks, other authors report different findings. Vathenen et al[252] showed that histamine

PD_{20} increased from 1.3 doubling doses to 2.4 doubling doses after 3 and 6 weeks respectively with 1600μg per day of budesonide. Furthermore, Juniper et al[423] showed that there was a strong treatment – time interaction with 400μg per day of budesonide in terms of methacholine bronchial challenge testing measured monthly for 1 year. The authors commented that the largest improvements occurred in the first 3 months but some patients have increasing bronchoprotection throughout the year. The tendency to reach a plateau was not related to asthma severity, age of onset or duration of therapy, or the use of steroids. However the patients studied all had mild asthma with an average FEV_1 of 90% predicted and only 3 out of 16 patients had ever received corticosteroid therapy.

Bernstein et al[424] showed improvements in symptoms scores and peak flow rate throughout a 6 week study of triamcinolone acetonide, however, there seemed to be a plateau after the 4th week. Furthermore, the effects of adenosine monophosphate challenge have been shown to occur much quicker. For example, Ketchell et al[425] showed that fluticasone propionate at a dose of 1000μg twice daily exhibited a 3.4 doubling dose shift in adenosine monophosphate bronchial challenge after 3 doses and a 3.75 doubling dose shift after 7 doses. Szefler et al[426] also showed statistical improvements with fluticasone propionate after 1 week and a maximum effect within 2 weeks for symptoms and rescue inhaler usage, and a maximum in FEV_1 within 3 weeks. However, had the treatments been given for longer durations in the studies in this thesis,

greater effects may have been found for the efficacy measures, especially when investigating inhaled corticosteroids.

All of the studies have been of crossover design rather than in parallel groups. This has been done in order to increase the statistical power, as each person acts as his or her own control. However, this design requires a washout period, which must be long enough for all endpoints to return to baseline. In the studies investigating the effects of corticosteroids on bronchial challenge testing (Chapter 8 and 9), a washout period of 1 week was used. Kraan et al[251] has shown that the effects of budesonide on methacholine return to baseline in 1 week. In all studies there was no significant difference between measurements after run-in and washout placebo periods. It is accepted statistical practice to use the mean of the two baselines or placebo periods in analysis with active treatment, as has been the case in this thesis.

10.12 Future studies arising from this thesis

There are still many unanswered questions which have been highlighted as a result of the work in this thesis. Although it was found that fluticasone propionate had greater effects on adrenal suppression than triamcinolone acetonide, the potency ratios for systemic activity of triamcinolone acetonide and fluticasone propionate still need to be calculated. This will require another dose-response study with higher doses of triamcinolone acetonide than evaluated in this thesis. As discussed previously, the use of

a spot morning urinary cortisol sample as a screening tool for adrenal suppression and the value of the low dose (0.5μg) ACTH stimulation test require further validation.

Many of the inhaled drugs investigated in this thesis were delivered by inhaler devices which used carboflurocarbons as the propellant. However as the production of chloro-fluro-carbons is now banned, new inhalers have been designed which contain hydrofluroalkanes as the propellant. These have been shown to alter the delivery characteristics of the aerosol[427] and therefore further studies require to be performed using these new inhalers.

New inhaled corticosteroids are also being developed such as mometasone furoate[428] or ciclesonide[359], and these drugs need to be investigated. There is also interest regarding the "non-genomic" properties of corticosteroids, such as the effect on nuclear factor-kappa B (see section 1.3.1), as these are thought to occur within minutes and be free from the genomic type systemic adverse effects. It is realised that corticosteroids have different proportions of genomic and non-genomic activity[429] and modifying their properties may be a way of increasing the therapeutic ratio of intra-nasal and inhaled corticosteroids.

The dose-response curves for systemic and efficacy effects for inhaled corticosteroids is dependant on dose (as seen in Chapter 6), lung delivery[171] and asthma severity[249]. Therefore each patient will have a unique dose with an optimal anti-inflammatory to

adverse effect ratio. Further studies are required to investigate methods of titrating the dose of inhaled corticosteroids on an individual patient basis in order to determine the optimal dose.

The optimal therapy for patients not controlled with low dose inhaled corticosteroids is yet to be determined. The last study in this thesis (Chapter 9) attempted to address this issue however it was a small pilot study and it should be repeated on a much larger basis, to further clarify this question. In the future, it may be possible to use genotype analysis in order to guide physicians as to the most appropriate medication. In this respect there is currently research looking at β_2 receptor[430] and the 5-lipoxygenase enzyme[431] polymorphisms.

This thesis has investigated the role of blocking the effects of leukotrienes when attempting to control airway inflammation. However, further studies are required to investigate the effects of leukotrienes in addition to other mediator blockers, such as anti-histamines. In the future there may be other inflammatory cytokine inhibitors which are licensed for allergic airways disease.

The studies in this thesis have evaluated anti-inflammatory medication used in allergic airway diseases, with the assumption that controlling inflammation will improve long term quality of life by controlling airway remodeling[260-262]. For example bronchial hyperresponsiveness induced by chronic inhaled allergen exposure in rats was shown to

be accompanied by smooth muscle hypertrophy, airway narrowing and goblet cell hyperplasia[432]. However, it is uncertain as to whether long-term unchecked inflammation leads to airways remodeling with airways obstruction[144]. Indeed further research is required to determine whether controlling inflammation is the key to controlling allergic airways disease in the long-term.

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12 PUBLICATIONS & PRESENTATIONS FROM THIS THESIS

12.1 Publications from this thesis

Wilson AM, McFarlane LC, Lipworth BJ. Dose response effect for adrenal suppression with repeated twice daily inhaled fluticasone propionate and triamcinolone acetonide in adult asthmatics. *American Journal of Respiratory and Critical Care Medicine* 1997; **156**: 1274-1277. (Chapter 3)

Wilson AM, McFarlane LC, Lipworth BJ. Effects of low and high doses of inhaled flunisolide and triamcinolone acetonide on basal and dynamic measures of HPA-axis activity in healthy volunteers. *Journal of Clinical Endocrinology and Metabolism* 1998; **83**: 922-925 (Chapter 3)

Wilson AM, Dempsey OJ, Coutie WJR, Sims EJ, Lipworth BJ. Importance of drug-device interaction in determining systemic effects of inhaled corticosteroids. *Lancet* 1999:353;2128 (Chapter 3)

Wilson AM, McFarlane LC, Lipworth BJ. Short-term dose-response relationships for relative systemic effects of oral prednisolone and inhaled fluticasone in asthmatic adults. *British Journal of Clinical Pharmacology* 1999;**48**:579-585 (Chapter 4)

Wilson AM, McFarlane LC, Lipworth BJ. Systemic bioactivity profiles of oral prednisolone and nebulised budesonide in adult astmatics. *Chest* 1998; **114**:1022-1027. (Chapter 4)

Wilson AM, McFarlane LC, Lipworth BJ. Effects of repeated once daily dosing of three intra-nasal corticosteroids on basal and dynamic measures of HPA-axis activity. *Journal of Allergy and Clinical Immunology* 1998; **101**: 470-74. (Chapter 5)

Wilson AM, McFarlane LC Lipworth BJ. Effects of intra-nasal corticosteroids on adrenal, bone and blood markers of systemic activity in allergic rhinitis. *Journal of Allergy and Clinical Immunology* 1998 **102**:598-604. (Chapter 5)

Wilson AM, McFarlane LC, Lipworth BJ. 24 Hour profiles of adrenocortical activity in asthmatic patients receiving inhaled and intra-nasal corticosteroids. *Thorax* 1999 **54**:20-27. (Chapter 5)

Wilson AM, Lipworth BJ. Dose response evaluation of the therapeutic index for inhaled corticosteroid therapy in asthmatic patients. *American Journal of Medicine* 2000; 108:269-275 (Chapter 6)

Wilson AM, Dempsey OJ, Sims EJ, Lipworth BJ. A comparison of topical budesonide and oral montelukast in seasonal allergic rhinitis and asthma. *Clinical Experimental Allergy* 2000 (Paper in press) (Chapter 7)

Aziz I, Wilson AM Lipworth BJ. Effects of once-daily formoterol and budesonide given alone or in combination on surrogate inflammatory markers in asthmatic adults. *Chest* 2000; 118:1049-1058 (Chapter 8)

Wilson AM, Dempsey OJ, Sims EJ, Lipworth BJ A comparison of salmeterol and montelukast as second-line therapy in asthmatic patients receiving inhaled corticosteroids. *Chest* (Paper in press). (Chapter 9)

12.2 Publications referred to in this thesis

Wilson AM, Clark DJ, McFarlane LC, and Lipworth BJ Adrenal suppression with high doses of inhaled fluticasone propionate and triamcinolone acetonide in healthy volunteers. *European Journal of Clinical Pharmacology* 1997 **53**: 33-37

Wilson AM, Brewster HA, and Lipworth BJ Dose response comparison of systemic bioactivity with inhaled budesonide and triamcinolone acetonide in asthmatic adults. *Journal of Allergy and Clinical Immunology* 1998;**102**: 751-756

Wilson AM, Clark DJ, Devlin M, McFarlane LC, and Lipworth BJ Adrenocortical activity with repeated administration of once daily inhaled fluticasone propionate and budesonide in asthamtic adults. *European Journal of Clinical Pharmacology* 1998 **53**: 317-320

Lipworth BJ, Wilson AM. Dose response to inhaled corticosteroids: benefits and risks. *Seminars in respiratory and critical care medicine.* 1998;**19**:625:642

Wilson AM, Sims EJ, Orr LC, Lipworth BJ. Differences in lung bioavailability between HFA and CFC formulations of fluticasone propionate. *Lancet* 1999;**354**:1357-1358

Dempsey OJ, Coutie WJR, Wilson AM, Williams P, Lipworth BJ Evaluation of the buccal component of systemic absorption with inhaled fluticasone propionate. *Thorax* 1999;**54**:614-617.

Dempsey OJ, Wilson AM, Coutie WJR, Lipworth BJ Evaluation of the effect of a large volume spacer on the systemic bioactivity of fluticasone propionate metered dose inhaler *Chest* 1999; 116:935-40.

Wilson AM, Dempsey OJ, Sims EJ, Coutie WJR, Paterson MC, Lipworth BJ Evaluation of treatment response in patients with seasonal allergic rhinitis using domiciliary nasal peak inspiratory flow. *Clinical and Experimental Allergy* 2000;30:833-838

Wilson AM, Blumsohn A, Jung RT, and Lipworth BJ. Asthma and cushings syndrome. *Chest* 2000; **117**:593-594

12.3 Presentations To Learned Societies

International

American Academy of Allergy, Asthma and Immunology

Lipworth BJ, Wilson AM. 24 hour adrenocortical profiles in asthmatics receiving inhaled and intra-nasal corticosteroids. Washington, March 1998 (*J Allergy Clin Immunol* 1998; **101** (Suppl Part 2): 58). Poster presentation.

Wilson AM, Sims EJ, Lipworth BJ, Optimisation of symptoms and lung function by corticosteroids may not equate with suppression of asthmatic inflammation. Orlando, March 1999. (*J Allergy Clin Immunol* 1999; **103** (Suppl Part 2): 62) Poster presentation

Aziz I, Wilson AM, Lipworth BJ. Effects of formoterol (FM) and budesonide (BUD), alone or in combination on adenosine monophosphate (AMP) challenge and exhaled nitric oxide (NO). Orlando, March 1999. (*J Allergy Clin Immunol* 1999; **103** (Suppl Part 2): 60)Poster presentation

American College of Allergy Asthma and Immunology

Lipworth BJ, Wilson AM. Effects of low and high doses of inhaled flunisolide and triamcinolone acetonide on basal and dynamic measures of HPA-axis activity. San Diego, November 1997 (*Ann Allergy Asthma Immunol* 1997; **76**:155). Oral Presentation.

Lipworth BJ, Wilson AM. Effects of repeated once daily dosing of intra-nasal corticosteroids on basal and dynamic measures of HPA-axis activity. San Diego, November 1997 (*Ann Allergy Asthma Immunol* 1997; **76**:155). Oral Presentation.

American Thoracic Society.

Lipworth BJ, Wilson AM, McFarlane LC. Evaluation of adrenal suppression with twice daily dosing of inhaled fluticasone propionate and triamcinolone acetonide in adult asthmatics. San Francisco May 1997. (*Am J Respir Crit Care Med* 1997 **155**(Suppl): A355). Poster presentation.

European Respiratory Society

Lipworth BJ, Wilson AM, McFarlane LC. Dose-response effects for adrenal suppression with twice repeated daily dosing of inhaled fluticasone propionate and triamcinolone acetonide in adult asthmatic patients. Berlin, September 1997 (*Eur Respir J* 1997; **10**(Suppl 25): 175S).Poster presentation.

Lipworth, BJ, Wilson AM, McFarlane, LC. The 24 hour profile with combined treatment of inhaled plus nasal fluticasone propionate (FP) and triamcinolone acetonide (TAA) in adult asthmatic patients. Geneva, September 1998. Poster presentation.

Wilson AM, Dempsey OJ, Sims EJ, Lipworth BJ. A comparison of salmeterol and montelukast as second-line therapy in asthmatic patients receiving inhaled corticosteroids. Madrid, Spain, October 1999.

Wilson AM, Lipworth BJ. Dose Reponse evaluation of the therapeutic index for inhaled

budesonide in atopic asthmatics. Madrid, Spain, October 1999

Paterson MC, Wilson AM, Dempsey OJ, Sims EJ, Lipworth BJ. The effect of combination therapy with salmeterol and montelukast in asthmatic patients receiving inhaled corticosteroid. Madrid, Spain, October 1999

Dempsey OJ, Wilson AM, Sims EJ, Lipworth BJ. A comparison of once daily topical budesonide and oral montelukast in seasonal allergic rhinitis and asthma.

National

British Pharmacological Society
Wilson AM, Lipworth BJ. A chronic dosing comparison of the systemic bioactivity with oral prednisolone and nebulised budesonide in adult asthmatic patients. Edinburgh September 1997 (*Br J Clin Pharmacol* in press). Oral presentation.

British Thoracic Society.
Wilson AM, McFarlane LC, Lipworth BJ, Adrenal suppression with high doses of inhaled fluticasone propionate and triamcinolone acetonide in normal subjects London December 1996 *Thorax* 1996; **51** (Suppl 3):A72). Poster presentation.

Wilson AM, Lipworth BJ. Systemic dose-response relationships with oral and inhaled corticosteroids in asthmatics London, December 1997 (*Thorax*, 1997; **52** (Suppl 6): A57) Poster presentation.

Wilson AM, Sims EJ, Lipworth BJ, Optimisation of symptoms and lung function by corticosteroids may not equate with suppression of asthmatic inflammation. London December 1998 Oral presentation

Caledonian Society Meeting
Wilson AM, Sims EJ, Lipworth BJ, Optimisation of symptoms and lung function by corticosteroids may not equate with suppression of asthmatic inflammation. Dundee October 1998 Oral presentation

Scottish Society for Experimental Medicine
Wilson AM, Lipworth BJ. Systemic dose-response relationships with oral and inhaled corticosteroids in asthmatics. Dundee November 1997 (*Scottish Medical Journal* in press). Oral presentation.

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Wilson AM, Sims EJ, Lipworth BJ, Optimisation of symptoms and lung function by corticosteroids may not equate with suppression of asthmatic inflammation. Dundee September 1998 Oral presentation

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Wilson AM, Lipworth BJ. Systemic tissue sensitivity for relative effects of oral and inhaled corticosteroids in asthmatic adults: a dose-response study. Creiff, June 1996. (*Scottish Medical Journal* in press). Oral presentation.

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Wilson AM, Dempsey OJ, Sims EJ, Lipworth BJ. A comparison of topical budesonide and oral montelukast in seasonal allergic rhinitis and asthma. Creif June 1999